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(54) Title: VECTORS HAVING ENHANCED EXPRESSION, AND METHODS OF MAKING AND USES THEREOF

## (57) Abstract

Disclosed and claimed are vectors having enhanced expression and methods for making and using them. Enhancement of expression is from substantially co-temporal expression of at least one first nucleic acid molecule and at least one second nucleic acid molecule. The second nucleic acid molecule encodes a transcription factor or a translation factor or a transcription factor and a translation factor. The contemporaneous expression can be from operably linking the first and second nucleic molecules to a single promoter, or from operably linking the first nucleic acid molecule to a first promoter and the second nucleic molecule to a second promoter wherein the first and second promoters function substantially contemporaneously. Thus, the first and second nucleic acid molecules can be at the same locus in the vector, or at different loci. The second nucleic acid molecule can encode: one transcription factor or more than one transcription factor; one translation factor or more than one translation factor; or at least one transcription factor and at least one translation factor. The transcription factor can be from vaccinia H4L, D6, A7, G8R, A1L, A2L, H5R, or combinations thereof. The translation factor can be from a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or combinations thereof. The vector can be a poxvirus such as an attenuated poxvirus, e.g., NYVAC, or ALVAC.

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TITLE OF THE INVENTION

VECTORS HAVING ENHANCED EXPRESSION,  
AND METHODS OF MAKING AND USES THEREOF

RELATED APPLICATIONS

5 Reference is made to the concurrently filed application of Tartaglia et al., "Vectors Having Enhanced Expression, And Methods of Making and Uses Thereof", Serial No. 08/815,809, incorporated herein by reference. Reference is also made to the copending applications of  
10 Paoletti et al., USSN 08/417,210, 08/303,275, 08/709,209, 08/184,009 (incorporating by reference USSN 07/805,567, from which U.S. Patent No. 5,378,457 issued) and 08/521,016 and to U.S. Patents Nos. 5,378,457, 5,225,336, 5,453,364, 5,494,807, 5,505,941, and 5,110,587, all of  
15 which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to enhanced vectors, and methods for making and using them. The vectors can have enhanced transcription or translation or  
20 enhanced transcription and translation and/or expression, e.g., enhanced transcription or translation or transcription and translation and/or expression from a nucleotide sequence of interest.

Several publications are referenced in this  
25 application. Full citation to these publications is found where cited or at the end of the specification, immediately preceding the claims or where the publication is mentioned; and each of these publications is hereby incorporated by reference. These publications relate to  
30 the state of the art to which the invention pertains; however, there is no admission that any of these publications is indeed prior art.

BACKGROUND OF THE INVENTION

DNA such as plasmids or naked DNA, and other  
35 vectors, such as viral vectors, e.g., vaccinia virus and more recently other poxviruses, have been used for the insertion and expression from foreign genes. The basic

technique of inserting foreign genes into live infectious  
poxvirus involves recombination between pox DNA sequences  
flanking a foreign genetic element in a donor plasmid and  
homologous sequences present donor plasmid and homologous  
5 sequences present in the rescuing poxvirus (Piccini et  
al., 1987). Recombinant poxviruses are constructed in  
steps known as in or analogous to methods in U.S. Patent  
Nos. 4,769,330, 4,772,848, 4,603,112, 5,505,941, and  
5,494,807, incorporated herein by reference. A desire in  
10 vector development is attenuated vectors, e.g., for  
enhanced safety; for instance, so that the vector may be  
employed in an immunological or vaccine composition.

For instance, the NYVAC vector, derived by  
deletion of specific virulence and host-range genes from  
15 the Copenhagen strain of vaccinia (Tartaglia et al.,  
1992) has proven useful as a recombinant vector in  
eliciting a protective immune response against an  
expressed foreign antigen. Likewise, the ALVAC vector, a  
vaccine strain of canarypox virus, has also proven  
20 effective as a recombinant viral vaccine vector (Perkus  
et al., 1995). In non-avian hosts, both these vectors do  
not productively replicate (with some exceptions as to  
NYVAC). Since all poxviruses replicate in the cytoplasm  
and encode most, if not all of the proteins required for  
25 viral transcription (Moss 1990), appropriately engineered  
foreign coding sequences under the control of poxvirus  
promoters are transcribed and translated in the absence  
of productive viral replication.

It would be an improvement over the state of  
30 the art to provide enhanced vectors, e.g., vectors having  
enhanced transcription or translation or transcription  
and translation and/or expression, for instance such  
vectors which are attenuated; especially since  
attenuation may raise issues of expression levels and/or  
35 persistence, and it would be an advancement to address  
such issues.

OBJECTS AND SUMMARY OF THE INVENTION

Recent studies on vaccinia replication have revealed certain poxvirus-encoded functions which play a role in the regulation of viral transcription and translation (reviewed in Moss, 1990; Moss, 1992). Some of these vaccinia encoded functions (e.g., E3L, K3L, H4L, and combinations thereof) have now surprisingly been utilized to increase the levels and persistence of gene expression (e.g., foreign gene expression) in vectors (e.g., the NYVAC and ALVAC vectors); and, are exemplary of the inventive vectors and methods.

Objects of the present invention may include at least one of: providing a method for increasing transcription or translation or transcription and translation and/or expression from at least one nucleotide sequence of interest by a vector, such as a coding nucleotide sequence by a vector; a vector having enhanced transcription or translation or transcription and translation; providing a method for preparing a vector having enhanced transcription or translation or transcription and translation and/or expression; providing a method for enhancing transcription or translation or transcription and translation and/or expression from a vector; providing an improved vector, such as poxvirus vectors, e.g., improved NYVAC, ALVAC or TROVAC vectors; and, products therefrom.

The invention thus provides a vector for enhanced expression of at least one first nucleotide sequence. The vector is modified to comprise at least one second nucleotide sequence encoding a transcription factor or translation factor or a transcription factor and a translation factor. The vector also can be modified to comprise the first nucleotide sequence. There is substantially co-temporal or substantially contemporaneous expression from the first and second nucleotide sequences. The expression is in a cell having a particular phenotype, and preferably the expression of

the first and second nucleotide sequences is with respect to the phenotype of the cell. Thus, expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation.

The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter, and the first and second promoters are preferably functional substantially co-temporally or contemporaneously. Thus, the first and second nucleotide sequences can be at different loci within the vector. The first and second nucleotide sequences also can be at the same locus within the vector, using the first and second promoters; or, by the first nucleotide sequence and the second nucleotide sequence being operably linked to a promoter.

The transcription factor can be of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof. The translation factor can effect inhibition of eIF-2 $\alpha$  phosphorylation or inhibition of PKR phosphorylation or otherwise sequester dsRNA which actually increases the concentration required to activate PKR. The translation factor can be selected from the group consisting of: a K3L open reading frame, an E3L open reading frame, a viral associated RNA I (VAI), an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

The first nucleotide sequence can be exogenous, e.g., encoding an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, a fusion protein or combinations thereof.

The vector can be a recombinant virus, such as a poxvirus; for instance, an orthopoxvirus or an avipoxvirus, e.g., a vaccinia virus, a fowlpox virus, a

canarypox virus; preferably an attenuated virus such as an attenuated poxvirus, e.g., NYVAC, ALVAC, or TROVAC.

The invention further provides a method for preparing a an inventive vector comprising modifying the  
5 vector to comprise the at least one second nucleotide sequence. The method can also include modifying the vector so that it comprises at the at least one first nucleotide sequence. Preferably the vector is so modified that there is substantially co-temporal or  
10 contemporaneous expression of the first and second nucleotide sequences; and, more preferably, the vector is also so modified that the expression is with respect to the phenotype of the cell. The method can comprise operably linking the first nucleotide sequence  
15 to a first promoter and the second nucleotide sequence to a second promoter, wherein the first and second promoters are functional substantially co-temporally or contemporaneously. The method can also comprise operably linking the first and second nucleotide sequences to a  
20 promoter.

The invention further provides an immunological, vaccine or therapeutic composition comprising at least one inventive vector and a pharmaceutically acceptable carrier or diluent.

25 The invention even still further provides a method for generating an immunological or therapeutic response in a host (animal, human, vertebrate, mammal, etc.) comprising administering to the host at least one inventive composition.

30 The invention additionally provides a method for increasing expression of at least one first nucleotide sequence by a vector comprising the first nucleotide sequence. The method comprises modifying the vector to comprise at least one second nucleotide  
35 sequence encoding a transcription factor or a translation factor or a transcription factor and a translation factor. There is preferably substantially co-temporal or

contemporaneous expression of the first and second nucleotide sequences. Expression can be in a cell having a particular phenotype; and it is more preferred to have expression be with respect to the phenotype of the cell.

- 5 Expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation. The method can additionally comprise modifying the vector to comprise the first nucleotide  
10 sequence of interest.

- The invention in yet another embodiment provides a method for expressing at least one gene product *in vitro* comprising infecting, or transfecting, a suitable cell with at least one inventive vector. The  
15 products therefrom can be an immunogen or epitope of interest, which can be useful in formulating therapeutic, immunological or vaccine compositions; or, for generating antibodies such as monoclonal antibodies; or, in assays, kits, tests and the like, such as diagnostic  
20 compositions, e.g., for detection of antibodies.

- Thus, the invention can provide compositions and methods for *in vitro* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for  
25 producing a gene product (which can be used as an immunogen or epitope in a therapeutic, immunological or vaccine composition, or in a diagnostic or detection kit, assay or method, e.g., to ascertain the presence or absence of antibodies, or to generate antibodies, such as  
30 monoclonal antibodies, e.g., for use in a diagnostic or detection kit, assay or method), and/or for *ex vivo* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene  
35 product for stimulating cells for reinfusion into a host (e.g., animal, mammal, vertebrate, human).

Additionally, in a further embodiment the



invention provides a method for expressing at least one nucleotide sequence (e.g., the at least one first nucleotide sequence) *in vivo* comprising administering at least one inventive vector to a host (human, animal, vertebrate, mammal, etc.). The nucleotide sequence can encode an immunogen or epitope of interest. The method can obtain antibodies. From generating antibodies one can generate monoclonal antibodies; or, antibodies are useful in assays, kits, tests or diagnostic compositions, e.g., for detection of antigens.

The invention can thus provide methods and compositions for *in vivo* transcription or translation or transcription and translation and/or expression involving the inventive vectors, e.g., administering at least one inventive vector or a composition comprising at least one inventive vector, for instance, therapeutic, immunological or vaccine compositions comprising at least one inventive vector and a suitable carrier or diluent (e.g., suitable for veterinary and human medicine).

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

#### **BRIEF DESCRIPTION OF THE FIGURES**

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

Fig. 1 shows the nucleotide sequence of the insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter (SEQ ID NO: );

Fig. 2 shows the nucleotide sequence of the ALVAC C8 Insertion site containing the H6/H42 expression cassette (SEQ ID NO: );

Fig. 3 shows the nucleotide sequence of the ALVAC C6 insertion site containing the H6/K3L and E3L expression cassette (SEQ ID NO: );

Fig. 4 shows the DNA sequence of the coding region of FHV gB with modified T5NT motifs (SEQ ID NO: );

Fig. 5 shows the DNA sequence of the H6 promoted FHV gB donor plasmid pC3H6FHVB (SEQ ID NO: );

Figs. 6 and 7 show DNA and amino acid sequences (SEQ ID NOS: ) of inserts in vCP1433 and vCP1452; and

Fig. 8 shows the DNA sequence (SEQ ID NO: ) of K3L E3L in vCP1452.

#### 10 DETAILED DESCRIPTION

U.S. Patent No. 5,494,807, to Paoletti et al., hereby incorporated herein by reference, relates to a modified recombinant virus having inactivated virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The viruses disclosed in Paoletti et al. can be poxviruses, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus, e.g., NYVAC, ALVAC and TROVAC. ALVAC was deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, USA, ATCC accession number VR-2547. TROVAC was likewise deposited under the terms of the Budapest Treaty with the ATCC, accession number 2553. And, NYVAC (vP866), vP994, vCP205, vCP1433, placZH6H4Lreverse, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, respectively.

Like the Paoletti et al. issued U.S. Patent, Falkner et al., WO 95/30018, published November 9, 1995, based on U.S. application 08/235,392, filed April 24, 1994 (both incorporated herein by reference), relates to poxviruses wherein loci for genetic functions associated with virulence (i.e., loci for "essential" functions) are employed for insertion of exogenous DNA.

Further, recombinants can be made from early (DNA<sup>-</sup>) and late defective mutants (see Condit and Niles,

"Orthopoxvirus Genetics," pp 1-39, In: Poxviruses, Edited by R. W. Moyer and P. C. Turner (Springer-Verlag, 1990), and documents cited therein, hereby incorporated herein by reference)), or from MVA which is said to be abortive late. Recombinants from defective mutants, abortive late viruses, viruses having essential genetic functions deleted or interrupted, or viruses having expression without productive replication (e.g., ALVAC in mammalian systems) may be said to be attenuated.

10 Certain vectors, such as attenuated vectors, e.g., NYVAC and ALVAC vectors, are blocked or limited in late gene expression in mammalian cells. Thus, early promoters are routinely employed in such vectors, e.g., NYVAC- or ALVAC-based recombinants, for expression from the  
15 foreign gene products.

Vaccinia encodes an open reading frame (ORF) designated H4L which has been shown to be required for early viral transcription (Ahn and Moss 1992, Zhang et al, 1994). The H4L ORF encodes an essential protein of  
20 94 kDa which is expressed after the start of viral DNA replication (late function). The H4L protein has been found to be tightly associated with the viral RNA polymerase complex and is believed to act in conjunction with the vaccinia early transcription factor (VETF) to  
25 initiate and transcribe early viral message (Ahn and Moss, 1992).

H4L is expressed late, but required early. This is consistent with the protein being packaged in the viral particles similar to that which is observed with  
30 VETF. This suggested that the amount of H4L present at early times post infection is low and perhaps limiting. Hence, one approach to increase foreign gene expression in an abortive, early vector-, e.g., virus-host interaction would be to increase the amount of H4L  
35 protein available during the early phase by expressing the H4L ORF using a vaccinia early/late promoter rather than the endogenous late promoter. Early expression from

H4L may not only increase the level of foreign gene transcripts, but also increase levels of other vaccinia early genes (e.g. E3L) which may also increase total protein levels.

5           There are other viral transcription factors; for instance, early and/or late viral transcription factors of poxvirus origin; e.g., from: vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, or vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X; see Kovacs  
10 et al., J. Virology, October 1996, 70(10):6796-6802, and documents cited therein, incorporated herein by reference). These and other transcription factors, and nucleotide sequences therefor or for homologs thereof, e.g., from another poxvirus, are useful in the practice  
15 of the invention.

          The selection of a suitable transcription factor is within the ambit of the skilled artisan from this disclosure and knowledge in the art; for instance, the skilled artisan can select a transcription factor  
20 based on an abortive phenotype of the vector, e.g., MVA is said to be abortive late, and a late or early or early/late transcription factor may be employed with this vector; ALVAC is abortive early and an early or early/late transcription factor may be employed with this  
25 vector; and, the vector can also be a ts (temperature sensitive) mutant (with respect to early (DNA<sup>-</sup>) and late defective mutants which can be also used in the practice of this invention, reference is made to Condit and Niles, *supra*). Thus, it is preferred that the transcription  
30 and/or translation factor and the at least one nucleotide sequence of interest be expressed early, late (including intermediate), or early/late, relative to the phenotype of the vector.

          Another means to increase foreign gene  
35 expression involves enhancing the overall efficiency of translation, e.g., mRNA translation, such as viral mRNA translation. Two vaccinia encoded functions (E3L and

K3L) have recently been identified as playing a role in the regulation of viral translation (Beattie et al., 1995a, 1995b, 1991; Chang et al., 1992; Davies et al., 1993). Both are capable of inhibiting the action of a cellular protein kinase (PKR) which, when activated by double stranded RNA (dsRNA), phosphorylates the translational initiation factor eIF-2 $\alpha$ , leading to an inhibition of initiation of mRNA translation (reviewed in Jacobs and Langland, 1996). Vaccinia virus, which produces dsRNA during viral transcription, has thus evolved mechanisms to block the negative action of PKR on eIF-2 $\alpha$  and allow for efficient translation of viral mRNA. (Asymmetric transcription gives rise to dsRNA; any viral infection or plasmid derived expression gives rise to it; dsRNA activates PKR; PKR becomes autophosphorylated, leading to phosphorylation of eIF-2 $\alpha$ .)

The vaccinia K3L ORF has been shown to have significant amino acid homology to eIF-2 $\alpha$  (Goebel et al., 1990; Beattie et al., 1991; U.S. Patent No. 5,378,457; see also Beattie et al., 1995a, 1995b). This protein is believed to act as a pseudosubstrate for PKR and competes for the eIF-2 $\alpha$  binding site (Carroll et al., 1993; Davies et al., 1992). The K3L gene product can bind to activated PKR and thus prevent phosphorylation of eIF-2 $\alpha$  with its resultant negative effect on translation initiation.

The vaccinia E3L gene codes for a protein which is capable of specifically binding to dsRNA (Watson and Jacobs, 1991; Chang et al., 1992). This would tend to lower the amounts of dsRNA in the infected cell, and thus reduce the level of activated PKR. When E3L was deleted from vaccinia, the resulting virus lost this kinase inhibitory function and further allowed activation of the 2' 5' oligoadenylate synthetase/RNase L pathway resulting in increased degradation of rRNA (Beattie et al., 1995a, 1995b). Thus, E3L appears to be critical for efficient mRNA translation in vaccinia infected cells at two

levels; mRNA stability and limiting eIF-2 $\alpha$  phosphorylation.

The ALVAC genome has been sequenced and searched for any homology to E3L/K3L or to any known  
5 dsRNA binding motif. Results have revealed no significant homology of any ALVAC ORFs to these two vaccinia ORFs, nor the presence of any dsRNA binding motifs.

Thus, an approach to improving expression  
10 levels in recombinant ALVAC vectors was to express the vaccinia E3L/K3L ORFs in ALVAC under the control of early vaccinia promoters. Through inhibition of PKR in the infected cells, the levels and persistence of foreign gene expression could be enhanced.

15 Hence, NYVAC and ALVAC recombinants as discussed herein were generated in order to enhance foreign gene expression at the transcriptional or translational or transcriptional and translational levels, as examples of the vectors and methods of the  
20 present invention.

Thus, exemplified herein is NYVAC recombinants having an early expressed H4L ORF and ALVAC recombinants having expression from the vaccinia E3L/K3L genes for enhancing or increasing the levels or persistence of  
25 expression of an inserted foreign gene. The up-regulation of foreign gene expression can have a profound effect on the induction of a therapeutic or immunological response in a host administered or inoculated with recombinants derived from these new vectors, thereby  
30 leading to an enhanced immunological, e.g., protective, response, or an enhanced therapeutic response.

The scope of the invention, i.e., to manipulate expression from any of a transcription and/or translation factor, e.g., H4L, E3L and K3L, to thereby enhance  
35 transcriptional or translational or transcriptional and translational and/or expression efficiency, can be extended to other eukaryotic vector systems (i.e. DNA,

viruses).

In fact, viruses in other families have also evolved mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation. In adenoviruses, the VAI RNA, transcribed by RNA pol III, has been well characterized and shown to bind directly to PKR, and thus, prevent its activation by dsRNA (Mathews and Shenk, 1991). Deletion of VAI from the adenovirus genome results in a mutant that replicates poorly and is deficient in levels of late gene expression (Thimmappaya et al., 1982). Similarly, Epstein-Barr virus, a herpesvirus, has an analogous RNA, called EBER, which also acts to prevent PKR activation by directly binding to the kinase (Clark et al., 1991; Sharp et al., 1993). The reovirus sigma 3 gene product has been shown to act in a similar manner as vaccinia E3L in binding dsRNA and thus preventing activation of PKR (Imani and Jacobs, 1988; see also Beattie et al. 1995a). Indeed, one study has shown that the reovirus sigma 3 gene can partially compensate a vaccinia recombinant deleted of E3L (Beattie et al., 1995a). Further, a cellular protein activated upon HIV infection (TRBP) has been shown to inhibit the activity of PKR (Park et al., 1994).

Thus, the present invention broadly relates to manipulation of expression, preferably by employing at least one transcription factor, e.g., at least one early and/or late viral transcription factor, or at least one translation factor, e.g., a nucleotide sequence encoding a product for overcoming the cellular anti-viral response of translational down-regulation through PKR activation in any eukaryotic vector system, or at least one transcription factor and at least one translation factor; for instance, to increase or enhance expression. And, the invention can pertain to any vector system, including, plasmid or naked DNA vectors, viral vectors, such as poxvirus, adenovirus, herpesvirus, baculovirus, and the like. Thus, the nucleotide sequences can be RNA

or DNA, for instance, as is suitable in view of the vector system.

Accordingly, the invention can relate to a vector modified to comprise at least one nucleotide sequence encoding at least one transcription factor, at least one translation factor, or at least one transcription factor and at least one translation factor; a method for increasing transcription and/or translation and/or expression by a vector or for preparing an inventive vector, e.g., by modifying the vector to comprise the at least one nucleotide sequence.

These methods can include substantially co-temporal expression from: (i) a first nucleotide sequence comprising at least one nucleotide sequence of interest, and (ii) a second nucleotide sequence comprising at least one nucleotide sequence encoding a transcription factor, or at least one nucleotide sequence encoding a translation factor or at least one nucleotide sequence encoding a transcription factor and a translation factor. The vector also can be modified to comprise the at least one nucleotide sequence of interest. The at least one nucleotide sequence of interest can be at least one coding nucleotide sequence. The vector preferably has substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences.

The substantially co-temporal expression can occur by employing promoters for the first and second nucleotide sequences which are functional at approximately the same time or stage of infection. Thus, the nucleotide sequence of interest and the nucleotide sequences encoding the factor(s) can be positioned at different loci in the vector. Alternatively, substantially co-temporal expression can occur by positioning the first and second nucleotide sequences within the same loci. Thus, substantially co-temporal expression can occur by operably linking to the nucleotide sequence of interest and/or to a promoter



operably linked to the nucleotide sequence of interest, a nucleotide sequence encoding a transcription factor, a nucleotide sequence encoding a translation factor, or a nucleotide sequence encoding a transcription factor and a translation factor.

The transcription factor can be from any suitable system. Preferably, the transcription factor is of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from expression from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, a homolog thereof and combinations thereof. It is also preferred that embodiments including a nucleotide sequence encoding a transcription factor comprise a poxvirus vector system.

The translation factor can likewise be from any suitable system. Preferably the translation factor can effect inhibition of eIF-2 $\alpha$  phosphorylation or inhibition of PKR phosphorylation or otherwise decreases cellular dsRNA content which increases the effective concentration of dsRNA. The translation factor can be selected from expression from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, a homolog thereof, and combinations thereof. The term "effective" with respect to dsRNA concentration means the amount of dsRNA to activate PKR and/or eIF-2 $\alpha$  phosphorylation (the dsRNA being in a form therefor). With respect to RNA-based factors, e.g., VAI RNA, EBER RNA, the skilled artisan can obtain suitable DNA from the RNA for use in a DNA vector system without undue experimentation. And, with respect to DNA-based factors, the skilled artisan can obtain suitable RNA therefrom for use in a RNA vector system, without undue experimentation.

The term "substantially co-temporal expression" or the term "substantially contemporaneous expression" means that the nucleotide sequence(s) encoding the

transcription or translation or transcription and translation factor(s) are expressed during approximately the same stage of infection as is the at least one nucleotide sequence of interest.

5 For instance, poxvirus genes are regulated in a temporal manner (Coupar, et al., Eur. J. Immunol., 1986, 16:1479-1487, at 1479). Thus, immediately after infection, a class of "early" genes is expressed (Id.). "Early genes" cease being expressed (i.e., early  
10 promoters cease functioning) at a time after infection prior to the "later" stage of infection (DNA replication commencement). The thymidine kinase ("TK") gene and TK promoter is an example of an immediate "early" gene and promoter (Hruby et al., J. Virol., 1982, 43(2):403-409,  
15 at 403). The TK gene is switched "off" about four hours after infection. "Late genes" are a class of genes not expressed until DNA replication has commenced (Coupar et al., *supra*). The PL11 promoter employed by Coupar et al. is an example of a "late" promoter. Thus,  
20 in Coupar et al., HA gene expression regulated by the PL11 promoter was not until after DNA replication, despite being in the TK region.

In contrast to canonical "early" genes and "late" genes the 7.5 kD gene and 7.5 kD promoter, is an  
25 example of an "early and late" gene and promoter. An "apparent exception to regulated transcription" (Davison and Moss, "Structure of Vaccinia Virus Early Promoters" J. Mol. Biol., 210-69, 249-69 (1989) at 749), the 7.5 kD promoter "contains regulatory signal for both early and  
30 late transcription" (Coupar et al., *supra*). Indeed, there are "independent early and late RNA start sites within the promoter region of the 7.5-kD gene" (Cochran et al., J. Virol., 59(1): 30-37 (April, 1985).

Coupar et al. observed "that temporal  
35 regulation of HA expression by the promoters PF [early], P7.5 [early and late] and PL11 [late] was maintained when the promoters were transposed to interrupt the TK gene of

[vaccinia virus]" (Id., at 1482). That is, Coupar et al. observed that foreign gene expression under the control of an early vaccinia promoter occurred "early", foreign gene expression under control of a late vaccinia promoter occurred "late", and foreign gene expression under the control of the early and late vaccinia 7.5 kD promoter occurred both early and late (See also id. at 1479: "[p]romoter sequences transposed to within the thymidine kinase (TK) gene continue to function in a temporally regulated manner" (citations omitted)).

Thus, the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be under the control of a first type of promoter and the at least one nucleotide sequence of interest or the coding nucleotide sequence can be under the control of a second type of promoter, wherein the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter can be early or late and the second promoter early and late; or the first promoter can be early and late and the second promoter early or late. The nucleotide sequence of interest and the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be at the same locus or at different loci; or under the control of the same promoter.

Accordingly, the invention can relate to a method for preparing a vector having enhanced transcription or translation or transcription and translation and/or expression, or to a method for increasing or enhancing transcription or translation or transcription and translation and/or expression in a vector comprising operably linking to at least one nucleotide sequence of interest, or to a promoter operably linked thereto, at least one nucleotide sequence for at least one transcription and/or at least one translation factor; e.g., at least one nucleotide

sequence for a transcription factor, or at least one nucleotide sequence for a translation factor or at least one nucleotide sequence for a transcription factor and a translation factor. Preferably the translation factor effects an inhibition of eIF-2 $\alpha$  phosphorylation and/or effects an inhibition of phosphorylation of PKR and/or a cellular kinase responsible for phosphorylation of eIF-2 $\alpha$  and/or effects the effective concentration of dsRNA. The invention also can thus relate to vectors from such methods.

Alternatively, the inventive methods can comprise operably linking at least one nucleotide sequence of interest to a first type of promoter and operably linking at least one second nucleotide sequence encoding at least one transcription and/or translation factor to a second type of promoter, within a vector, wherein the first and second promoters are both functional at the same time or same stage of infection, e.g., the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter is early or late and the second promoter is early and late; or the first promoter is early and late and the second promoter is early or late. Of course, the first and second promoters can be the same promoter at two or more different loci, or the same promoter at one locus. And, the invention thus relates to vectors from such methods.

And, the term "nucleotide sequence" as used herein can mean nucleic acid molecule. Thus, a nucleotide sequence can be an isolated nucleic acid molecule, e.g., exogenous DNA.

Accordingly, the present invention can provide vectors modified to contain at least one exogenous nucleotide sequence, preferably encoding at least one epitope of interest, and at least one transcription factor or at least one translation factor or at least one transcription factor and at least one translation factor,

wherein there is substantially temporal co-expression (or substantially co-temporal expression or substantially contemporaneous expression) of the exogenous nucleotide sequence and the factor(s); and, methods for making and using such vectors and products therefrom. Enhanced or improved expression is obtained by the vectors and methods of the invention; and, enhanced or improved expression can mean enhanced levels and/or persistence of expression.

10           The invention can thus provide vectors, for instance, poxvirus vectors, which are abortive early, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the levels and/or persistence of an inserted nucleotide sequence, e.g., a foreign gene. The invention can also provide vectors, for instance, poxvirus vectors, which are abortive late (which includes abortive intermediate), e.g., MVA recombinants, having a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) open reading frame (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the levels and/or persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

          The invention can additionally provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having expression from the vaccinia E3L and/or K3L (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus,

plasmid or naked DNA, and the like, note discussion supra of viral mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation) as a means for enhancing and/or increasing the levels and persistence of an inserted nucleotide sequence, e.g., a foreign gene.

Even further still, the invention can provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof) and/or a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) (or a homolog thereof), for instance abortive late (which includes abortive intermediate), e.g., MVA, recombinants, and expression from the vaccinia E3L and/or K3L (or a homolog thereof) as a means for enhancing and/or increasing the levels and persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

As shown in the Examples below, ALVAC-HIV recombinant vCP1452 containing the K3L/E3L factors had enhanced expression on human cells in comparison to vCP1433 or vCP300. Indeed, enhanced expression is observed with the E3L/K3L translational factors in human and canine cells.

Enhanced expression by translational factors such as E3L/K3L may be cell type dependent. For instance, while enhanced expression with E3L/K3L is observed in human and canine cells it is not observed in murine and feline cells. From this disclosure and the knowledge in the art, the skilled artisan can select an appropriate translational factor for use with a particular cell type, without undue experimentation. For example, it should go without saying that the skilled artisan knows the differences between cells. Thus it is preferred that the translational factor be expressed in a cell in which enhanced expression is observed, e.g., that

the translational factor employed be with respect to the cell.

Further, preliminary immunogenicity studies in mice show no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no observed enhanced expression in murine cells. Accordingly, the skilled artisan from this disclosure and the knowledge in the art can select a translational factor which will provide enhanced immunogenicity in a desired animal, without undue experimentation. If enhanced expression is observed *in vitro* in a particular cell line by a particular translational factor, e.g., E3L/K3L in human or canine cells, the skilled artisan can thus expect enhanced immunogenicity *in vivo* in the animal (including human) from which the cells were derived by that particular translational factor, e.g., enhanced immunogenicity in humans and canines from the E3L/K3L translational factor.

Furthermore, in murine cells, the limiting factor of ALVAC expression is at the transcription level. Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be a factor in *in vitro* or *in vivo* applications of the invention (note H4 data), as may be the nature of the vector, e.g., the phenotype of the vector; but, appropriate selection of a cell and vector phenotype and of time of expression from factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and the knowledge in the art, without undue experimentation.

Also, the Examples below show that NYVAC recombinant vP1380 has enhanced expression levels in comparison to vP994. Possibly, part of the enhanced levels in vP1380 are due to enhanced transcription and expression from viral specific products such as E3L, such that there is enhanced transcription and translation

involved in expression in vP1380. There is more expression from the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and  
5 more persistent levels of expression.

Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994. Another observation is that enhancement profiles are seen  
10 in restrictive early cells in the abortive early NYVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication, e.g., VERO or CEF, suggesting that it may be preferred that the factor and the foreign DNA be expressed  
15 substantially co-temporally or contemporaneously, i.e., that preferably there be co-expression at substantially the same time or stage, and that the time of expression, e.g., early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early,  
20 abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an  
25 abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and the transcriptional or translational or transcriptional and translational factor(s) early; in an abortive late system, one preferably expresses exogenous DNA and the  
30 transcriptional or translational or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).

35 The methods for making a vector or recombinant can be by or analogous to the methods disclosed in U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941,



5,338,683, 5,494,807, and 4,722,848; WO 95/30018, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al., "Production of Human Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, U.S. application Serial No. 920,197, filed October 16, 1986, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman, "The function of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143, Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et

al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also U.S. applications Serial Nos. 08/675,566 and 08/675,556, relating to vectors, including adenovirus vectors.

10 As to the inserted nucleic acid molecule in a vector of the invention, e.g., the foreign gene, the heterologous or exogenous nucleic acid molecule, e.g., DNA, in vectors of the instant invention, preferably encodes an expression product comprising: an epitope of  
15 interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene or a fusion protein. With respect to these terms, reference is made to the following discussion, and generally to Kendrew, THE ENCYCLOPEDIA OF MOLECULAR BIOLOGY (Blackwell  
20 Science Ltd., 1995) and Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1982.

An epitope of interest is an immunologically relevant region of an antigen or immunogen or  
25 immunologically active fragment thereof, e.g., from a pathogen or toxin of veterinary or human interest.

An epitope of interest can be prepared from an antigen of a pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the  
30 pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a canine distemper virus or measles or rinderpest antigen such as HA or F; a rabies glycoprotein, e.g., rabies glycoprotein  
35 G; an avian influenza antigen, e.g., turkey influenza HA, Chicken/Pennsylvania/1/83 influenza antigen such as a nucleoprotein (NP); a bovine leukemia virus antigen, e.g.,

gp51,30 envelope; a Newcastle Disease Virus (NDV) antigen, e.g., HN or F; a feline leukemia virus antigen (FeLV), e.g., FeLV envelope protein; RAV-1 env; matrix and/or preplomer of infectious bronchitis virus; a  
5 Herpesvirus glycoprotein, e.g., a glycoprotein from feline herpesvirus, equine herpesvirus, bovine herpesvirus, pseudorabies virus, canine herpesvirus, HSV, Marek's Disease Virus, or cytomegalovirus; a flavivirus antigen, e.g., a Japanese encephalitis virus (JEV)  
10 antigen, a Yellow Fever antigen, or a Dengue virus antigen; a malaria (*Plasmodium*) antigen, an immunodeficiency virus antigen, e.g., a feline immunodeficiency virus (FIV) antigen or a simian immunodeficiency virus (SIV) antigen or a human  
15 immunodeficiency virus antigen (HIV); a parvovirus antigen, e.g., canine parvovirus; an equine influenza antigen; an poxvirus antigen, e.g., an ectromelia antigen, a canarypox virus antigen or a fowlpox virus antigen; or an infectious bursal disease virus antigen,  
20 e.g., VP2, VP3, VP4.

An epitope of interest can be from an antigen of a human pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits  
25 a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a measles virus antigen such as HA or F; a rabies glycoprotein, e.g., rabies virus glycoprotein G; an influenza antigen, e.g., influenza virus HA or N; a Herpesvirus antigen, e.g., a  
30 glycoprotein of a herpes simplex virus (HSV), a human cytomegalovirus (HCMV), Epstein-Barr; a flavivirus antigen, a JEV, Yellow Fever virus or Dengue virus antigen; a Hepatitis virus antigen, e.g., HBsAg; an immunodeficiency virus antigen, e.g., an HIV antigen such  
35 as gp120, gp160; a Hantaan virus antigen; a *C. tetani* antigen; a mumps antigen; a pneumococcal antigen, e.g., PspA; a *Borrelia* antigen, e.g., OspA, OspB, OspC of

*Borrelia* associated with Lyme disease such as *Borrelia burgdorferi*, *Borrelia afzelli* and *Borrelia garinii*; a chicken pox (varicella zoster) antigen; or a *Plasmodium* antigen.

5           Of course, the foregoing lists are intended as exemplary, as the epitope of interest can be derived from any antigen of any veterinary or human pathogen; and, to obtain an epitope of interest, one can express an antigen of any veterinary or human pathogen (such that the  
10 invention encompasses the exogenous or foreign nucleic acid molecule(s) of interest encoding at least one antigen).

          Since the heterologous DNA can be a growth factor or therapeutic gene, the inventive recombinants  
15 can be used in gene therapy. Gene therapy involves transferring genetic information; and, with respect to gene therapy and immunotherapy, reference is made to U.S. Patent No. 5,252,479, which is incorporated herein by reference, together with the documents cited in it and on  
20 its face, and to WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also incorporated herein by reference, together with the documents cited therein. The growth factor or therapeutic gene, for example, can encode a disease-  
25 fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-  
30 globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin, macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma  
35 associated antigen or B7.

          The invention further relates to an immunogenic, immunological or vaccine composition

containing the inventive vector and an acceptable carrier or diluent (e.g., veterinary acceptable or pharmaceutically acceptable). An immunological composition containing the vector (or an expression product thereof) elicits an immunological response - local or systemic. The response can, but need not be protective. An immunogenic composition containing the inventive recombinants (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The invention therefore also provides a method for inducing an immunological response in a host vertebrate comprising administering to the host an immunogenic, immunological or vaccine composition comprising the inventive recombinant virus or vector and an acceptable carrier or diluent. For purposes of this specification, "animal" includes all vertebrate species, except humans; and "vertebrate" includes all vertebrates, including animals (as "animal" is used herein) and humans. And, of course, a subset of "animal" is "mammal", which for purposes of this specification includes all mammals, except humans.

For human administration, the inventive recombinants or vectors, can provide the advantage of expression without productive replication. This thus provides the ability to use recombinants of the invention in immunocompromised individuals; and, provides a level of safety to workers in contact with recombinants of the invention. Therefore, the invention comprehends methods for amplifying or expressing a protein by administering or inoculating a host with a recombinant virus or vector, whereby the host is not a natural host of the recombinant

virus or vector, and there is expression without productive replication.

The exogenous or heterologous DNA (or DNA foreign to vaccine virus) can be DNA encoding any of the  
5 aforementioned epitopes of interest, as listed above. In this regard, with respect to *Borrelia* DNA, reference is made to U.S. Patent No. 5,523,089, WO93/08306, PCT/US92/08697, *Molecular Microbiology* (1989), 3(4), 479-486, and PCT publications WO 93/04175, and WO 96/06165,  
10 incorporated herein by reference.

With respect to pneumococcal epitopes of interest, reference is made to Briles et al. WO 92/14488, incorporated herein by reference, with respect to tumor viruses reference is made to Molecular Biology of Tumor  
15 Viruses, RNA TUMOR VIRUSES (Second Edition, Edited by Weiss et al., Cold Spring Harbor Laboratory 1982) (e.g., page 44 et seq. - Taxonomy of Retroviruses), incorporated herein by reference.

With respect to DNA encoding epitopes of  
20 interest, attention is directed to documents cited herein, see, e.g., documents cited *supra* and documents cited *infra*, for instance: U.S. Patents Nos. 5,174,993 and 5,505,941 (e.g., recombinant avipox virus, vaccinia virus; rabies glycoprotein (G), gene, turkey influenza  
25 hemagglutinin gene, gp51,30 envelope gene of bovine leukemia virus, Newcastle Disease Virus (NDV) antigen, FeLV envelope gene, RAV-1 env gene, NP (nucleoprotein gene of Chicken/Pennsylvania/1/83 influenza virus), matrix and preplomer gene of infectious bronchitis virus; HSV gD),  
30 U.S. Patent No. 5,338,683 (e.g., recombinant vaccinia virus, avipox virus; DNA encoding Herpesvirus glycoproteins, *inter alia*), U.S. Patent No. 5,494,807 (e.g., recombinant vaccinia, avipox; exogenous DNA encoding antigens from rabies, Hepatitis B, JEV, YF,  
35 Dengue, measles, pseudorabies, Epstein-Barr, HSV, HIV, SIV, EHV, BHV, HCMV, canine parvovirus, equine influenza, FeLV, FHV, Hantaan, *C. tetani*, avian influenza, mumps,

NDV, *inter alia*), U.S. Patent No. 5,503,834 (e.g., recombinant vaccinia, avipox, Morbillivirus, e.g., measles F, hemagglutinin, *inter alia*), U.S. Patent No. 4,722,848 (e.g., recombinant vaccinia virus; HSV tk, HSV glycoproteins, e.g., gB, gD, influenza HA, Hepatitis B, e.g., HBsAg, *inter alia*), U.K. Patent GB 2 269 820 B and U.S. Patent No. 5,514,375 (recombinant poxvirus; flavivirus structural proteins); WO 92/22641 and U.S. applications Serial Nos. 08/417,210 and 08/372,664 (e.g., recombinant poxvirus; immunodeficiency virus, HTLV, *inter alia*), WO 93/03145 and allowed U.S. applications 08/204,729 and 08/303,124 (e.g., recombinant poxvirus; IBDV, *inter alia*), WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994 (e.g., recombinant poxvirus; cytokine and/or tumor associated antigens, *inter alia*), U.S. application Serial No. 08/469,969 (rabies combination compositions), U.S. application Serial No. 08/746,668 (lentivirus, retrovirus and/or immunodeficiency virus, including feline immunodeficiency virus, *inter alia*), U.S. Patent No. 5,529,780 and allowed U.S. application Serial No. 08/413,118 (canine herpesvirus), U.S. application Serial No. 08/471,025 (calicivirus), WO 96/3941 and U.S. application Serial No. 08/658,665 (cytomegalovirus), and PCT/US94/06652 (*Plasmodium* antigens such as from each stage of the *Plasmodium* life cycle).

As to antigens for use in vaccine or immunological compositions, reference is made to the documents and discussion set forth in the documents cited herein (see, e.g., documents cited *supra*); see also Stedman's Medical Dictionary (24th edition, 1982, e.g., definition of vaccine (for a list of antigens used in vaccine formulations; such antigens or epitopes of interest from those antigens can be used in the invention, as either an expression product of an inventive recombinant virus or vector, or in a multivalent composition containing an inventive

recombinant virus or vector or an expression product therefrom).

As to epitopes of interest, one skilled in the art can determine an epitope or immunodominant region of a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation.

A general method for determining which portions of a protein to use in an immunological composition focuses on the size and sequence of the antigen of interest. "In general, large proteins, because they have more potential determinants are better antigens than small ones. The more foreign an antigen, that is the less similar to self configurations which induce tolerance, the more effective it is in provoking an immune response." Ivan Roitt, Essential Immunology, 1988.

As to size: the skilled artisan can maximize the size of the protein encoded by the DNA sequence to be inserted into the viral vector (keeping in mind the packaging limitations of the vector). To minimize the DNA inserted while maximizing the size of the protein expressed, the DNA sequence can exclude introns (regions of a gene which are transcribed but which are subsequently excised from the primary RNA transcript).

At a minimum, the DNA sequence can code for a peptide at least 8 or 9 amino acids long. This is the minimum length that a peptide needs to be in order to stimulate a CD8+ T cell response (which recognizes virus infected cells or cancerous cells). A minimum peptide length of 13 to 25 amino acids is useful to stimulate a CD4+ T cell response (which recognizes special antigen presenting cells which have engulfed the pathogen). See Kendrew, *supra*. However, as these are minimum lengths, these peptides are likely to generate an immunological



response, i.e., an antibody or T cell response; but, for a protective response (as from a vaccine composition), a longer peptide is preferred.

With respect to the sequence, the DNA sequence preferably encodes at least regions of the peptide that generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest "is fragmented into overlapping peptides with proteolytic enzymes. The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since the T cell recognizes short linear peptides complexed with MHC molecules. The method is less effective for determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, (1992) pp. 79-80.

Another method for determining an epitope of interest is to choose the regions of the protein that are hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, (1992) p. 81

Yet another method for determining an epitope of interest is to perform an X-ray crystallographic analysis of the antigen (full length)-antibody complex. Janis Kuby, Immunology, (1992) p. 80.

Still another method for choosing an epitope of interest which can generate a T cell response is to identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be

presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be  
5 considered are: the HLA type of the patient (vertebrate, animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

10 An immune response is generated, in general, as follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex MHC" located on another cell's surface. There are two  
15 classes of MHC complexes - class I and class II, and each class is made up of many different alleles. Different patients have different types of MHC complex alleles; they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually  
20 every cell and present peptides from proteins produced inside the cell. Thus, Class I MHC complexes are useful for killing cells which when infected by viruses or which have become cancerous and as the result of expression of an oncogene. T cells which have a protein called CD8 on  
25 their surface, bind specifically to the MHC class I/peptide complexes via the T cell receptor. This leads to cytolytic effector activities.

Class II MHC complexes are found only on antigen- presenting cells and are used to present  
30 peptides from circulating pathogens which have been endocytosed by the antigen- presenting cells. T cells which have a protein called CD4 bind to the MHC class II/peptide complexes via the T cell receptor. This leads to the synthesis of specific cytokines which stimulate an  
35 immune response.

Some guidelines in determining whether a protein is an epitopes of interest which will stimulate a

T cell response, include: Peptide length - the peptide should be at least 8 or 9 amino acids long to fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MHC complex. This length is  
5 a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut the expressed peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or  
10 class II molecules with high enough specificity to generate an immune response (See Bocchia, M. et al, Specific Binding of Leukemia Oncogene Fusion Protein Peptides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with  
15 class I and class II MHC molecules Ann. Rev. Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Protein epitopes  
20 recognized by T cell receptors are peptides generated by enzymatic degradation of the protein molecule and are presented on the cell surface in association with class I or class II MHC molecules.

Further, the skilled artisan can ascertain an  
25 epitope of interest by comparing the protein sequence with sequences listed in the protein data base.

Even further, another method is simply to generate or express portions of a protein of interest, generate monoclonal antibodies to those portions of the  
30 protein of interest, and then ascertain whether those antibodies inhibit growth *in vitro* of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in this disclosure and in the art for generating or  
35 expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth *in vitro*. For example, the skilled artisan can generate portions of

a protein of interest by: selecting 8 to 9 or 13 to 25 amino acid length portions of the protein, selecting hydrophylic regions, selecting portions shown to bind from X-ray data of the antigen (full length)-antibody complex, selecting regions which differ in sequence from other proteins, selecting potential HLA anchor binding motifs, or any combination of these methods or other methods known in the art.

Epitopes recognized by antibodies are expressed on the surface of a protein. To determine the regions of a protein most likely to stimulate an antibody response one skilled in the art can preferably perform an epitope map, using the general methods described above, or other mapping methods known in the art.

As can be seen from the foregoing, without undue experimentation, from this disclosure and the knowledge in the art, the skilled artisan can ascertain the amino acid and corresponding DNA sequence of an epitope of interest for obtaining a T cell, B cell and/or antibody response. In addition, reference is made to Gefter et al., U.S. Patent No. 5,019,384, issued May 28, 1991, and the documents it cites, incorporated herein by reference (Note especially the "Relevant Literature" section of this patent, and column 13 of this patent which discloses that: "A large number of epitopes have been defined for a wide variety of organisms of interest. Of particular interest are those epitopes to which neutralizing antibodies are directed. Disclosures of such epitopes are in many of the references cited in the Relevant Literature section.")

With respect to expression of a biological response modulator, reference is made to Wohlstadter, "Selection Methods," WO 93/19170, published 30 September 1993, and the documents cited therein, incorporated herein by reference.

For instance, a biological response modulator modulates biological activity; for instance, a biological

response modulator is a modulatory component such as a high molecular weight protein associated with non-NMDA excitatory amino acid receptors and which allosterically regulates affinity of AMPA binding (See Kendrew, *supra*).

- 5 The recombinant of the present invention can express such a high molecular weight protein.

More generally, nature has provided a number of precedents of biological response modulators. Modulation of activity may be carried out through mechanisms as  
10 complicated and intricate as allosteric induced quaternary change to simple presence/absence, e.g., expression/degradation, systems. Indeed, the repression/activation of expression of many biological molecules is itself mediated by molecules whose  
15 activities are capable of being modulated through a variety of mechanisms.

Table 2 of Neidhardt et al *Physiology of the Bacterial Cell* (Sinauer Associates Inc., Publishers, 1990), at page 73, lists chemical modifications to  
20 bacterial proteins. As is noted in that table, some modifications are involved in proper assembly and other modifications are not, but in either case such modifications are capable of causing modulation of function. From that table, analogous chemical  
25 modulations for proteins of other cells can be determined, without undue experimentation.

In some instances modulation of biological functions may be mediated simply through the proper/improper localization of a molecule. Molecules  
30 may function to provide a growth advantage or disadvantage only if they are targeted to a particular location. For example, a molecule may be typically not taken up or used by a cell, as a function of that molecule being first degraded by the cell by secretion of  
35 an enzyme for that degradation. Thus, production of the enzyme by a recombinant can regulate use or uptake of the molecule by a cell. Likewise, the recombinant can

express a molecule which binds to the enzyme necessary for uptake or use of a molecule, thereby similarly regulating its uptake or use.

5       Localization targeting of proteins carried out through cleavage of signal peptides another type of modulation or regulation. In this case, a specific endoprotease catalytic activity can be expressed by the recombinant.

10       Other examples of mechanisms through which modulation of function may occur are RNA virus poly-proteins, allosteric effects, and general covalent and non-covalent steric hindrance. HIV is a well studied example of an RNA virus which expresses non-functional poly-protein constructs. In HIV "the gag, pol, and env  
15 poly-proteins are processed to yield, respectively, the viral structural proteins p17, p24, and p15--reverse transcriptase and integrase--and the two envelope proteins gp41 and gp120" (Kohl et al., PNAS USA 85:4686-90 (1988)). The proper cleavage of the poly-proteins is  
20 crucial for replication of the virus, and virions carrying inactive mutant HIV protease are non-infectious (*Id.*). This is another example of the fusion of proteins down-modulating their activity. Thus, it is possible to construct recombinant viruses which express molecules  
25 which interfere with endoproteases, or which provide endoproteases, for inhibiting or enhancing the natural expression of certain proteins (by interfering with or enhancing cleavage).

30       The functional usefulness of enzymes may also be modulated by altering their capability of catalyzing a reaction. Illustrative examples of modulated molecules are zymogens, formation/disassociation of multi-subunit functional complexes, RNA virus poly-protein chains, allosteric interactions, general steric hindrance  
35 (covalent and non-covalent) and a variety of chemical modifications such as phosphorylation, methylation, acetylation, adenylation, and uridylation (see Table 1

of Neidhardt, *supra*, at page 315 and Table 2 at page 73).

Zymogens are examples of naturally occurring protein fusions which cause modulation of enzymatic activity. Zymogens are one class of proteins which are converted into their active state through limited proteolysis. See Table 3 of Reich, *Proteases and Biological Control*, Vol. 2, (1975) at page 54). Nature has developed a mechanism of down-modulating the activity of certain enzymes, such as trypsin, by expressing these enzymes with additional "leader" peptide sequences at their amino termini. With the extra peptide sequence the enzyme is in the inactive zymogen state. Upon cleavage of this sequence the zymogen is converted to its enzymatically active state. The overall reaction rates of the zymogen are "about  $10^5$ - $10^6$  times lower than those of the corresponding enzyme" (See Table 3 of Reich, *supra* at page 54).

It is therefore possible to down-modulate the function of certain enzymes simply by the addition of a peptide sequence to one of its termini. For example, with knowledge of this property, a recombinant can express peptide sequences containing additional amino acids at one or both termini.

The formation or disassociation of multi-subunit enzymes is another way through which modulation may occur. Different mechanisms may be responsible for the modulation of activity upon formation or disassociation of multi-subunit enzymes.

Therefore, sterically hindering the proper specific subunit interactions will down-modulate the catalytic activity. And accordingly, the recombinant of the invention can express a molecule which sterically hinders a naturally occurring enzyme or enzyme complex, so as to modulate biological functions.

Certain enzyme inhibitors afford good examples of functional down-modulation through covalent steric hindrance or modification. Suicide substrates which

irreversibly bind to the active site of an enzyme at a catalytically important amino acid in the active site are examples of covalent modifications which sterically block the enzymatic active site. An example of a suicide  
5 substrate is TPCK for chymotrypsin (Fritsch, *Enzyme Structure and Mechanism*, 2d ed; Freeman & Co. Publishers, 1984)). This type of modulation is possible by the recombinant expressing a suitable suicide substrate, to thereby modulate biological responses (e.g., by limiting  
10 enzyme activity).

There are also examples of non-covalent steric hindrance including many repressor molecules. The recombinant can express repressor molecules which are capable of sterically hindering and thus down-modulating  
15 the function of a DNA sequence by preventing particular DNA-RNA polymerase interactions.

Allosteric effects are another way through which modulation is carried out in some biological systems. Aspartate transcarbamoylase is a well  
20 characterized allosteric enzyme. Interacting with the catalytic subunits are regulatory domains. Upon binding to CTP or UTP the regulatory subunits are capable of inducing a quaternary structural change in the holoenzyme causing down-modulation of catalytic activity. In  
25 contrast, binding of ATP to the regulatory subunits is capable of causing up-modulation of catalytic activity (Fritsch, *supra*). Using methods of the invention, molecules can be expressed which are capable of binding and causing modulatory quaternary or tertiary changes.

30 In addition, a variety of chemical modifications, e.g., phosphorylation, methylation, acetylation, adenylation, and uridylation may be carried out so as to modulate function. It is known that modifications such as these play important roles in the  
35 regulation of many important cellular components. Table 2 of Neidhardt, *supra*, at page 73, lists different bacterial enzymes which undergo such modifications. From



that list, one skilled in the art can ascertain other enzymes of other systems which undergo the same or similar modifications, without undue experimentation. In addition, many proteins which are implicated in human  
5 disease also undergo such chemical modifications. For example, many oncogenes have been found to be modified by phosphorylation or to modify other proteins through phosphorylation or dephosphorylation. Therefore, the ability afforded by the invention to express modulators  
10 which can modify or alter function, e.g., phosphorylation, is of importance.

From the foregoing, the skilled artisan can use the present invention to express a biological response modulator, without any undue experimentation.

15 With respect to expression of fusion proteins by inventive recombinants, reference is made to Sambrook, Fritsch, Maniatis, *Molecular Cloning, A LABORATORY MANUAL* (2d Edition, Cold Spring Harbor Laboratory Press, 1989) (especially Volume 3), and Kendrew, *supra*, incorporated  
20 herein by reference. The teachings of Sambrook et al., can be suitably modified, without undue experimentation, from this disclosure, for the skilled artisan to generate recombinants or vectors expressing fusion proteins.

With regard to gene therapy and immunotherapy,  
25 reference is made to U.S. Patent Nos. 4,690,915 and 5,252,479, which are incorporated herein by reference, together with the documents cited therein it and on their face, and to WO 94/16716 and U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also  
30 incorporated herein by reference, together with the documents cited therein.

A growth factor can be defined as multifunctional, locally acting intercellular signalling peptides which control both ontogeny and maintenance of  
35 tissue and function (see Kendrew, *supra*, especially at page 455 et seq.).

The growth factor or therapeutic gene, for

example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin (e.g., an interleukin selected from interleukins 1 to 14, or 1 to 11, or any combination thereof), macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7. U.S. Patent No. 5,252,479 provides a list of proteins which can be expressed in an adenovirus system for gene therapy, and the skilled artisan is directed to that disclosure. WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, provide genes for cytokines and tumor associated antigens and immunotherapy methods, including ex vivo methods, and the skilled artisan is directed to those disclosures.

Thus, one skilled in the art can create recombinants or vectors expressing a growth factor or therapeutic gene and use the recombinants or vectors, from this disclosure and the knowledge in the art, without undue experimentation.

Moreover, from the foregoing and the knowledge in the art, no undue experimentation is required for the skilled artisan to construct an inventive recombinant or vector which expresses an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, or a fusion protein; or for the skilled artisan to use such a recombinant or vector.

As the recombinants or vectors of the invention can be used for expression of gene products *in vitro*,

techniques for protein purification can be employed in the practice of the invention, and such techniques, in general, include:

Briefly, the cells are disrupted and the protein of interest is released into an aqueous "extract". There are many methods of cellular disintegration, which vary from relatively gentle to vigorous conditions, and the choice of one method over the other is dependent upon the source material. Animal tissues vary from the very easily broken erythrocytes to tough collagenous material such as found in blood vessels and other smooth-muscle containing tissue. Bacteria vary from fairly fragile organisms that can be broken up by digestive enzymes or osmotic shock to more resilient species with thick cell walls, needing vigorous mechanical treatment for disintegration.

Gentle techniques include cell lysis, enzymatic digestion, chemical solubilization, hand homogenization and mincing (or grinding); moderate techniques of cell disintegration include blade homogenization and grinding with abrasive materials, i.e., sand or alumina; and vigorous techniques include french press, ultrasonication, bead mill or Manton-Gaulin homogenization. Each of the aforementioned techniques are art-recognized, and it is well within the scope of knowledge of the skilled artisan to determine the appropriate method for cell disintegration based upon the starting material, and the teachings herein and in the art.

Following cell disintegration, the extract is prepared by centrifuging off insoluble material. At this stage, one may proceed with the purification method, as an extract containing as much of the protein of interest as possible has been prepared, and, where appropriate, particulate and most nonprotein materials have been removed.

Standard techniques of protein purification may

be employed to further purify the protein of interest, including: precipitation by taking advantage of the solubility of the protein of interest at varying salt concentrations, precipitation with organic solvents, polymers and other materials, affinity precipitation and selective denaturation; column chromatography, including high performance liquid chromatography (HPLC), ion-exchange, affinity, immuno affinity or dye-ligand chromatography; immunoprecipitation and the use of gel filtration, electrophoretic methods, ultrafiltration and isoelectric focusing. Each of the above-identified methods are well within the knowledge of the skilled artisan, and no undue experimentation is required to purify the proteins or epitopes of interest from expression of a recombinant or vector of the invention, using the standard methodologies outlined hereinabove, and in the literature, as well as the teachings in the Examples below.

As the expression products can provide an antigenic, immunological, or protective (vaccine) response, the invention further relates to products therefrom; namely, antibodies and uses thereof. More in particular, the expression products can elicit antibodies by administration of those products or of recombinants or vectors expressing the products. The antibodies can be monoclonal antibodies; and, the antibodies or expression products can be used in kits, assays, tests, and the like involving binding, so that the invention relates to these uses too. Additionally, since the recombinants or vectors of the invention can be used to replicate DNA, the invention relates to the inventive recombinants as vectors and methods for replicating DNA by infecting or transfecting cells with the recombinant and harvesting DNA therefrom. The resultant DNA can be used as probes or primers or for amplification.

The administration procedure for the inventive recombinants or vectors or expression products thereof,

compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response. The administration can be via a mucosal route, e.g., oral, nasal, genital, etc. Such an administration enables a local immune response.

More generally, the inventive antigenic, immunological or vaccine compositions or therapeutic compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical, medical or veterinary arts. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered with other compositions of the invention or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or epitopes from expression by an inventive recombinant or vector or another vector system; and are administered taking into account the aforementioned factors.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, genital, e.g., vaginal, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant or vector may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or

the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the recombinant or vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al. J. Immunol. 147:410-415 (1991) and incorporated by reference herein, encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, NH) can also be used.

The composition may be packaged in a single dosage form for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if the recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host, as well as LD<sub>50</sub> and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 µg. The inventive recombinant or vector can be administered in any suitable amount to achieve

expression at these dosage levels. The viral recombinants of the invention can be administered in an amount of about  $10^{3.5}$  pfu; thus, the inventive viral recombinant is preferably administered in at least this amount; more preferably about  $10^4$  pfu to about  $10^6$  pfu; however higher dosages such as about  $10^4$  pfu to about  $10^{10}$  pfu, e.g., about  $10^5$  pfu to about  $10^9$  pfu, for instance about  $10^6$  pfu to about  $10^8$  pfu can be employed. Suitable quantities of inventive plasmid or naked DNA in plasmid or naked DNA compositions can be 1 ug to 100 mg; preferably 0.1 to 10 mg, but lower levels such as 0.1 to 2 mg or preferably 1-10 ug may be employed. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or recombinant or vector may be lyophilized for resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nanoparticles, reported by Kreuter, J., Microcapsules and Nanoparticles in Medicine and Pharmacology, M. Donbrow (Ed). CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are

polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,l-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al. Current Topics in Microbiology and Immunology, 1989, 146:59-66. The entrapment of antigens in PLGA microspheres of 1 to 10 microns in diameter has been shown to have a remarkable adjuvant effect when administered orally. The PLGA microencapsulation process uses a phase separation of a water-in-oil emulsion. The compound of interest is prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of an assortment of agents (polyvinyl alcohol (PVA), gelatin, alginates, polyvinylpyrrolidone (PVP), methyl cellulose) and the solvent removed by either drying in vacuo or solvent extraction.

Thus, solid, including solid-containing-liquid, liquid, and gel (including "gel caps") compositions are envisioned.

Furthermore, the inventive vectors or recombinants can be used in any desired immunization or



administration regimen; e.g., as part of periodic vaccinations such as annual vaccinations as in the veterinary arts or as in periodic vaccinations as in the human medical arts, or as in a prime-boost regimen

5 wherein an inventive vector or recombinant is administered either before or after the administration of the same or of a different epitope of interest or recombinant or vector expressing such a same or different epitope of interest (including an inventive recombinant  
10 or vector expressing such a same or different epitope of interest), see, e.g., documents cited herein such as U.S. application Serial No. 08/746,668.

Additionally, the inventive vectors or recombinants and the expression products therefrom can  
15 stimulate an immune or antibody response in animals. From those antibodies, by techniques well-known in the art, monoclonal antibodies can be prepared and, those monoclonal antibodies, can be employed in well known antibody binding assays, diagnostic kits or tests to  
20 determine the presence or absence of antigen(s) and therefrom the presence or absence of the natural causative agent of the antigen or, to determine whether an immune response to that agent or to the antigen(s) has simply been stimulated.

25 Monoclonal antibodies are immunoglobulin produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides greater specificity than a conventional, serum-derived antibody. Furthermore, screening a large number of  
30 monoclonal antibodies makes it possible to select an individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant, inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily  
35 standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No.

4,196,265, issued Apr. 1, 1989, incorporated herein by reference.

Uses of monoclonal antibodies are known. One such use is in diagnostic methods, e.g., David, G. and  
5 Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983, incorporated herein by reference.

Monoclonal antibodies have also been used to recover materials by immunoadsorption chromatography, e.g. Milstein, C., 1980, Scientific American 243:66, 70,  
10 incorporated herein by reference.

Furthermore, the inventive recombinants or vectors or expression products therefrom can be used to stimulate a response in cells *in vitro* or *ex vivo* for subsequent reinfusion into a patient. If the patient is  
15 seronegative, the reinfusion is to stimulate an immune response, e.g., an immunological or antigenic response such as active immunization. In a seropositive individual, the reinfusion is to stimulate or boost the immune system against a pathogen.

20 The recombinants or vectors of the invention are also useful for generating DNA for probes or for PCR primers which can be used to detect the presence or absence of hybridizable DNA or to amplify DNA, e.g., to detect a pathogen in a sample or for amplifying DNA.

25 Since viruses require translation of viral mRNAs in order to generate viral proteins required for replication, it is evident that any function which blocks the action of PKR in the infected cell will have a positive effect on viral protein expression. Thus, co-  
30 expression, in some fashion, of the vaccinia E3L/K3L gene products, or a homolog of E3L and/or K3L, may provide a general mechanism for enhancing the expression levels of heterologous gene products by vectors in general. The E3L/K3L or homologous functions may enhance or augment  
35 native anti-PKR mechanisms, and thus increase protein expression levels and/or persistence. This provides a useful element towards optimizing the efficiency of

eukaryotic virus systems as immunization vehicles. This approach could be further extended for improvement of DNA-based immunogens, e.g., naked DNA or plasmid DNA vector systems. Further, employing a nucleotide sequence for a transcription factor, e.g., for an early and/or late viral transcription factor, in conjunction with enhancing translation by employing a nucleotide sequence for a translation factor, can even further enhance or increase expression by increasing or enhancing transcription and translation; and thus, increasing or enhancing levels or persistence of expression can be obtained.

A better understanding of the present invention and of its many advantages will be had from the following non-limiting Examples, given by way of illustration.

#### EXAMPLES

##### Example 1 - NYVAC Recombinants Containing H4L

The plasmids placZH6H4L and placZH6H4Lreverse (ATCC Deposit No. \_\_\_\_\_) were used as donor plasmids for in vivo recombination with the rescue virus vP994 (ATCC Deposit No. \_\_\_\_\_; U.S. Patent No. 5,494,807, incorporated herein by reference; vaccinia H6 promoter/HIV1 MN env-noncleavable, secreted gp140, in HA insertion site). The donor plasmids were designed to replace the endogenous promoter and coding sequences of H4L by homologous recombination. The resulting recombinant viruses were designated vP1379 and vP1380; vP1379 contains the H6lacZ/H6H4L cassette in a head-to-head configuration; vP1380 contains the H6lacZ/H6H4L cassette in a head-to-tail configuration (SEQ ID NO: \_\_\_\_\_; Fig. 1).

The plasmids were constructed as follows:

##### H4L Expression Cassette

The H4L open reading frame (orf) as delineated in Goebel et al. 1990 corresponds to positions 94830-92446 in the Copenhagen (vaccine) strain vaccinia virus genomic sequence. pSD404VC contains a clone of the 8.6Kb

*Hind*III H fragment of Copenhagen vaccinia virus inserted into the pUC vector background. pSD404VC was digested with *Pvu*II to isolate a 3860bp fragment containing the H4L coding sequences and flanking sequences. The 3860bp  
5 fragment was inserted into the blunted *Bam*HI site of pBSecogpt (*E.coli* gpt gene (ATCC No. 37145) under the control of Copenhagen B13R promoter in the pBS SK vector (Stratagene La Jolla, CA.)) resulting in plasmid pRW935.

pRW935 was linearized with *Eco*RI and partially  
10 digested with *Dra*I to remove a 970bp fragment containing the 5' end of the H4L coding sequence. Using a series of Polymerase Chain Reactions (PCRs) the H4L coding sequence was reengineered to be under the control of the modified vaccinia H6 promoter (Perkus et al. 1989). Using the  
15 plasmid template pRW935 and primer pairs RW500/RW502 and RW501/RW503 in the PCR amplifications, the 5' H4L sequences were regenerated. In addition, the oligonucleotide, RW502, modifies the H4L coding sequences (position 341-348 from the A of the ATG) from TTTTTTTT to  
20 TTTTCTTC without altering the predicted amino acid sequence to remove an early transcriptional stop signal (Yuan, L. and Moss, B., 1987). The modified H6 promoter was amplified from the plasmid template pRW936 using oligonucleotides RW504 and RW507. Oligonucleotides RW505  
25 and RW506 having complementary sequences were PCR amplified directly. The four PCR reactions were pooled and further amplified using primer pair RW500 and RW505. The resulting PCR fragment was digested with *Dra*I and *Eco*RI and cloned into *Dra*I and *Eco*RI digested pRW935  
30 generating pRW939. A PCR introduced error in the 5' end of the coding region of pRW939 was corrected, resulting in plasmid pRW947. Specifically, the PCR error introduced in pRW939 (H4L codon 155 is AAA - correct codon should be GAA) was corrected by replacement of the  
35 600 bp pRW939 *Afl*III-*Eco*RI fragment with the equivalent fragment from pRW935 to generate pRW939. The oligonucleotide sequences for each of the above-

identified oligonucleotides (RW500 and RW501 to RW507;

SEQ ID NOS: ) are:

RW500 5' - GAAATAGTTAGCGTCAAC -3'

RW501 5' - TGTCTAATGTGTTGAAGAAAAGATCATAACAAGTTATAC -3'

5 RW502 5' - AACTTGTATGATCTTTTCTTCAACACATTAGACATGTATTTAC -3'

RW503 5' - TAAGTTTGTATCGTAATGGACTCTAAAGAGACTATTC -3'

RW504 5' - AGTCTCTTTAGAGTCCATTACGATACAACTTAAC -3'

RW505

5' -

10 CCGACGATTTTAAACGCCACCGTCAGGGAAAGTTTCATAAGAAGCACCGGAAGAGA  
AGAGA ATTCTCGGGACAATTGGATC -3'

RW506

5' -

GTCTAGCTGGTGCTGAGTTTCTACGTGAGTTGATTTCGTCTCTTGCGTGCCTCTCGTG

15 ATCCAATTGTCCCGAGATATTCTC -3'

RW507

5' -

GTAGAAACTCAGCACCAAGCTAGACAAGCTTCTTTATTCTATACTTAAAAAGTGAAAA  
TAAATAC -3'

20 The plasmid pRW947 was digested with *Xho*I to  
generate two fragments. The 7036 bp fragment containing  
the H6 promoted H4L in the pBSSK vector background was  
purified and self-ligated, resulting in the plasmid  
pH6H4L. The plasmid pRW973A, containing a LacZ  
25 expression cassette under the control of the vaccinia H6  
promoter, was digested with *Hind*III. The 3.3 Kbp  
fragment was purified and ligated into the *Hind*III  
digested pH6H4L, thereby generating pLACZH6H4Lreverse  
(H6 promoted LacZ gene and H6 promoted H4L gene in head-  
30 to-tail configuration), and placZH6H4L (H6 promoted lacZ  
gene and H6 promoted H4L gene in a head-to-head  
configuration).

#### Example 2 - ALVAC Recombinants

35 pMPC6H6K3E3 (ATCC No. \_\_\_\_ ) was used as a donor  
plasmid in *in vivo* recombination (Piccini et al., 1987)  
with rescuing virus vCP205 (ATCC No. \_\_\_\_; U.S.  
application Serial No. 08/417,210, incorporated herein by

reference; HIV expression cassette - vaccinia H6 promoter/HIV truncated env MN strain, I3L gag with protease in ALVAC C3 insertion site); and the resulting recombinant virus was designated vCP1431A (vaccinia H6/K3L and E3L cassette in the C6 locus).

pC8H6H4 was used as the donor plasmid in *in vivo* recombination with vCP205 and the resulting recombinant virus designated vCP1435 (HIV cassette at C3 locus and the vaccinia H6/H4L expression cassette at C8 locus; H6/H4L expression cassette flanked by ALVAC C8 insertion site sequences (SEQ ID NO: ) shown in Fig. 2).

vCP1431A was also used as a rescuing virus in *in vivo* recombination using plasmid pC8H6H4, generating the recombinant designated vCP1437A (HIV cassette at the C3 locus, the H6/K3L and E3L cassette at the C6 locus, and the vaccinia H6/H4L cassette at the C8 locus). With respect to the H6/K3L expression cassette and the vaccinia E3L gene with the endogenous promoter flanked by the ALVAC C6 insertion site sequences reference is made to Fig. 3 (SEQ ID NO: ).

pC3H6FHVB (ATCC No. \_\_\_\_; Fig. 5, SEQ ID NO: ; H6 promoted FHV gB ORF with early transcriptional and translational stop signals at both 5' and 3' ends flanked by the left and right arms of the ALVAC C3 locus) was used in *in vivo* recombination with the ALVAC (ATCC No. VR-2547) to generate vCP1459 (H6 promoted FHV gB expression cassette in deorfed C3 insertion locus). With respect to the FHV-1 gB coding region in which the two internal T<sub>5</sub>NT motifs have been mutated, see Fig. 4 (SEQ ID NO: ).

pC3H6FHVB was used in *in vivo* recombination with vCP1431A to generate vCP1460 (H6 promoted FHV gB expression cassette in the deorfed C3 insertion locus and vaccinia E3L/K3L genes in C6 locus).

pC3H6FHVB was used in *in vivo* recombination with vCP1437 to generate vCP1464 (H6 promoted FHV gB

expression cassette in deorfed C3 insertion locus, vaccinia E3L/K3L genes in C6 locus and H6 promoted vaccinia H4L ORF in C8 locus).

pMPC5H6PN (HIV pol/nef "string of beads" cassette in the ALVAC C5 locus) was used in recombination with vCP205 to obtain vCP1433 (ATCC Deposit No. \_\_\_\_). Thus, recombinant ALVAC-MN120TMGNPst (vCP1433) was generated by insertion of an expression cassette encoding a synthetic polypeptide containing all of the known Pol CTL epitopes (Nixon and McMichael; 1991) and all of the known human Nef CTL epitopes into vCP205 at the insertion site known as C5.

pMPC6H6K3E3 (ATCC Deposit No. \_\_\_\_ ; containing vaccinia H6/K3L expression cassette and vaccinia E3L gene with endogenous promoter flanked by the ALVAC C6 insertion site sequences) was used in recombination with vCP1433 to obtain vCP1452. Figures 6 and 7 show the nucleotide and amino acid sequences of the vCP1433 and vCP1452 inserts. Figure 8 shows the K3L E3L in C6 in vCP1452. vCP1452 contains the HIV type 1 gag and protease genes derived from the IIIB isolate, the gp120 envelope sequences derived from the MN isolate, and sequences encoding a polypeptide encompassing the known human CTL epitopes from HIV-1 Nef and Pol (Nef1 and Nef2 CTL epitopes, and Pol1, Pol2 and Pol3 CTL epitopes). The expressed gp120 moiety is linked to the transmembrane (TM) anchor sequence (28 amino acids) of the envelope glycoprotein. In addition to the HIV coding sequences vCP1452 contains the vaccinia virus E3L and K3L coding sequences inserted into the C6 site. The insertion sites and promoter linkages for this construct are shown in the Table below.

Table: Insertion sites and promoter linkages in vCP1452

| Insert                                  | Insertion Site | Promoter   |
|---|----------------|------------|
| HIV1 MN gp120 + TM                      | C3             | H6         |
| HIV1 IIIB gag (+ pro)                   | C3             | I3L        |
| 5 Pol3/Nef C term/Pol2/Nef central/Pol1 | C5             | H6         |
| Vaccinia virus E3L                      | C6             | endogenous |
| Vaccinia virus K3L                      | C6             | H6         |

10 vCP300 is an ALVAC recombinant containing HIV gp120TM (MN), gag/pro (IIIB) (C3 locus), Nef (C6 locus), and Pol (C5 locus), as described in U.S. application Serial No. 08/417,210, incorporated herein by reference.

15 Plasmids for preparing these recombinants were prepared as follows:

Vaccinia H4L Expression Cassette Into ALVAC

pCPM6LDEL was generated by using primer pair H4A and H4B to amplify a 900 bp fragment from pBAMM11.6 (ALVAC 11.6kb BamHI M fragment in pBSSK bector background), and primer pair H4C and H4D (SEQ ID NOS: ) to amplify a 940 bp fragment from pBAMM11.6 (H4C 5'ACTACTAATTAGCTATAAAAACCCGGGATTAGTTTTTATTACTAACTAATTACTA TACTG3') (H4D 5'ATCATCGGATCCTTTAATAATCTTATGAACTTTTATAAATATGAG3'). A fusion PCR reaction using the PCR products from the amplifications and primer pair H4A and H4D obtained an 1840 bp PCR fusion fragment which was then cloned into the T/A Cloning vector for sequence confirmation. The sequence was found to have a PCR deletion at position 8054. The 1840 bp fragment was removed from the T/A vector by digestion with BamHI. The fragment was then cloned into the BamHI digested pBSSK ΔEcoRI-SmaI vector. The deletion was repaired by digesting the construct with HindIII to remove a 250 bp fragment of the right arm and 35 religating to obtain pCPM6LDEL.



placZH6H4Lreverse was digested with *PspAI* and *Asp700* resulting in a 1920bp fragment containing the H6 promoter and the 5' 1780bp of the H4L gene. The remaining 590bp of the H4L gene were generated using PCR amplification from the plasmid template placZH6H4Lreverse using primer pair H4A and H4B. The oligonucleotide sequences for primer pair H4A and H4B (SEQ ID NOS: ) are:

#### Oligonucleotide Sequences

- 10 H4A 5' - ATCATCGAAGAGCTTCCGCTATCTGCATTAAAGTTT-3' ..  
H4B 5' - ATCATCCCCGGAAGCTTTTAGTTATTGAAATTAATCATATA-3'

The 590bp PCR fragment was gel purified and cloned into the TA Cloning vector (Invitrogen San Diego, CA. 92121) for sequence confirmation. The 590bp insert containing the 3' H4L sequences was excised from the TA vector by digestion with *PspAI* and *Asp700*. The 1920bp and the 590bp fragments were directionally cloned into the *PspAI* digested pCPM6LDEL plasmid vector (containing the deorfed ALVAC M6L insertion site) to generate the plasmid pM6LDELH6H4 containing the H6/H4L expression cassette flanked by ALVAC sequences at the M6L insertion site.

ALVAC pC8 insertion vector was generated as follows: PCR J36, containing the C8 ORF and flanking sequences, was generated using JP121 (CAT-CAT-GAG-CTC-  
25 ACT-TAT-TAC-ATC-CTA-CT) and JP122 (TAC-TAC-GGT-ACC-TTT-AAT-AAG-CAA-TCA-CT)

(SEQ ID NOS: ) on ALVAC DNA. The resulting approximately 1.7 kb band was digested with *Asp718/SacI* and ligated into *Asp718/SacI* digested pBSSK+. After  
30 confirmation by sequence analysis, the resulting plasmid was designated pCPF85S3L. To remove most of the C8 ORF and introduce transcriptional and translational stops along with a MCS into pCPF85S3L, the plasmid was digested with *SnaBI/HindIII* and ligated to -115bp PCR J618I  
35 *SnaBI/HindIII* fragment, yielding pC8. PCR J618I is a fusion PCR product of PCRs J616 and J617 using primers JP516 (TAG-GAA-GAT-ACG-TAT-TAT-TTT-ATA-C) and JP519 (ATC-

CCA-TTA-TGA-AAG-CTT-ATA-G). PCR J616 was generated using primers JP516 and JP517 (CTC-GAG-CTG-CAG-GAT-ATC-ATC-GAT-GGA-TCC-TTT-TTA-TAG-CTA-ATT-AGT-CAC-GTA-CCT-TTA-TCA-TTA-GTA-ACA-AAT) on plasmid pCPF85S3L. PCR J617 was  
5 generated using primers JP518 (GGA-TCC-ATC-GAT-GAT-ATC-CTG-CAG-CTC-GAG-TTT-TTA-TGA-CTA-GTT-AAT-CAC-GGC-CGC-TCA-ATA-TTG-TAT-TGG-ATG-GTT-AG) and JP519 on plasmid pCPF85S3L. Plasmid pC8, the C8 insertion plasmid, was confirmed by sequence analysis and contains a ~440bp left  
10 arm, a ~1162bp right arm, a MCS with unique *Bam*HI, *Cla*I, *Eco*RV, *Pst*I, and *Xho*I sites, flanked by both transcriptional and translational stop sequences.

From the plasmid pM6LDELH6H4, the 2.5 Kbp H6/H4 expression cassette was excised with *Sma*I, and the  
15 resulting 2.5 Kbp *Sma*I fragment was purified and inserted into the ALVAC pC8 insertion vector at the *Eco*RV site generating pC8H6H4.

#### K3L Expression Cassette

The K3L coding sequences were synthesized by  
20 PCR amplification using pSD407VC containing Copenhagen vaccinia HindIII K fragment as template, as described in U.S. Patent No. 5,378,457. The oligonucleotides MPSYN 763 and MPSYN 764 (SEQ ID NOS: ) were used as primers for the PCR reaction.

25 MPSYN 763

5' -

CCCTCTAGATCGCGATATCCGTTAAGTTTGTATCGTAATGCTTGCAATTTTGTTATTC  
GT-3'

MPSYN 764 5' - CCCGAATTCATAAAAATTATTGATGTCTACA-3'

30 The approximately 325bp PCR fragment was digested with *Xba*I and *Eco*RI yielding a 315bp fragment. This 315bp fragment was purified by isolation from an agarose gel and ligated with *Xba*I and *Eco*RI digested pBSSK+ vector (from Stratagene LA Jolla, CA.). The  
35 nucleic acid sequence was confirmed directly from alkali denatured plasmid template as described in Hattori, M. and Sakaki, Y., 1986, using the modified T7 polymerase

(Tabor, S. and Richardson, C.C. 1987) and Sequenase (from U.S. Biochemicals Cleveland, OH.). This plasmid was designated pBS 763/764. Digesting pBS 763/764 with *Nru*I and *Xho*I, a 340bp fragment was isolated for cloning into the plasmid vector pMM154 containing a cassette with the vaccinia H6 promoter controlling an irrelevant gene in the NYVAC *tk* insertion vector background, which was prepared by digestion with *Nru*I (partially) and *Xho*I, such that the 340bp fragment from pBS 763/764 containing the K3L gene could be directionally oriented next to the H6 promoter generating pMPTKH6K3L. The plasmid pMP42GPT containing the dominant selectable marker *Eco* gpt gene (Pratt D. and Subramani S. 1983) under the control of the Entomopox 42k promoter, was digested with *Sma*I and *Bam*HI to yield a 0.7 Kbp 42k-*Eco* gpt expression cassette. This 0.7 Kbp fragment was purified and ligated into *Sma*I and *Bam*HI cut pMPTKH6K3L generating the plasmid pMPTKH6K3Lgpt. This plasmid was digested with *Xho*I, generating a 1.2 Kbp fragment containing the H6/K3L and the 42k/*Ecogpt* expression cassette, which was then gel purified. The 1.2 Kbp *Xho*I fragment was inserted into the *Xho*I site of the ALVAC C6 insertion plasmid pC6L (described in U.S. Patent No. 5,494,807), generating pMPC6H6K3Lgpt.

#### 25 E3L/K3L ALVAC Expression Cassette

The entire E3L gene is contained within a 2.3 Kbp *Eco*RI fragment isolated from pSD401VC, which contained a clone of the *Hind*III E fragment from Copenhagen vaccinia. The 2.3 Kbp *Eco*RI fragment was inserted into pMPC6H6K3Lgpt that had been partially digested with *Eco*RI, generating the plasmid pMPC6H6K3E3gpt. The plasmid pMPC6H6K3E3gpt was digested with *Xho*I and the resulting 6.8 Kbp vector fragment was purified and self-ligated, resulting in the plasmid pMPC6E3. The plasmid pMPTKH6K3L was digested with *Psp*AI and the resulting 560bp fragment containing the H6/K3L expression cassette was ligated into *Psp*AI digested

pMPC6E3 resulting in the plasmid construct pMPC6H6K3E3.

Construction of the H6-promoted FHV gB donor plasmid

The entire coding region of the Feline Herpesvirus 1 glycoprotein gB (FHV-1 gB) was obtained by  
5 digestion of pJCA079 (FHV gB coding region in which 5' and 3' T<sub>5</sub>NT sequences were mutated to change the early transcriptional stop signal without affecting amino acid sequences; the I3L vaccinia promoter has been coupled to the 5' end of the gB ORF; see Fig. 4, SEQ ID NO:) with  
10 *Pst*I and isolating a 3 Kbp fragment from an agarose gel. The purified *Pst*I fragment was cloned into an ALVAC C3 insertion plasmid (pVQH6CP3LSA) also digested with *Pst*I (the unique *Bam*HI site in pVQH6CP3LSA was previously inactivated by digestion with *Bam*HI, blunting the ends  
15 with Klenow polymerase and religation; pVQH6CP3LSA was obtained by digesting pVQH6CP3L, discussed in U.S. Patent No. 5,494,807, with *Not*I and *Nsi*I, from which a 6623 bp fragment was isolated and ligated to annealed oligonucleotides CP34 (5'GGCCGCGTCGACATGCA3') and CP35  
20 (5'TGTCGACGC3') (SEQ ID NOS: ). The resulting plasmid, pRAC5, was screened for proper orientation of the gB coding region with respect to the H6 promoter. To properly link the H6 promoter to the FHV gB initiation codon, an 800 bp PCR fragment was amplified from pJCA079  
25 using oligonucleotides RG789 (SEQ ID NO: ) (5'-TTTCATTATCGCGATATC-CGTTAAGTTTGTATCGTAATGTCCACTCGTGGCGATC-3') and RG787 (SEQ ID NO: ) (5'-GGAGGGTTTCAGAGGCAG-3'). This purified fragment was digested with *Nru*I/*Bam*HI and ligated into pRAC5 also digested with *Nru*I/*Bam*HI. The  
30 resulting plasmid was the FHV gB donor plasmid, pC3H6FHVb.

"String of Beads" Cassette

The "string of beads" expression cassette for the *nef* and *pol* CTL epitopes (H6/Pol 3/Nef C term/Pol  
35 2/Nef central/Pol 1) was generated by PCR (polymerase chain reaction) as detailed below, using template pHXBD2 for *pol* epitopes and template 2-60-HIV.3 for *Nef*

epitopes. Initial assembly was in two parts: (1) H6(partial promoter)/Pol 3/Nef C term(Nef 2); (2) Pol 2/Nef central (Nef 1)/Pol 1 in pBSSK. These were combined, then moved to pBSH6-11 for the assembly of the entire H6 promoter, then the H6/HIV cassette was moved to a C5 insertion plasmid.

(1) H6/Pol 3/Nef C term(Nef 2)

A 230 bp fragment (A) was derived by PCR to obtain the H6 linkage and Pol3 using synthetic oligonucleotides MPSYN783 and MPSYN784 and template pHXBD2. pHXBD2 was derived at the NIH/NCI (Dr. Nancy Miller) from a recombinant phage library of XbaI digested DNA from HTLV-III infected H9 cells cloned in lambda-J1 (Shaw et al., 1994). This plasmid contains the entire proviral DNA sequence of the HIV IIIB isolate.

A 110 bp fragment (B) was derived by PCR to obtain Nef2 using oligonucleotides MPSYN785/MPSYN786 and template p2-60-HIV.3 (described in U.S. application Serial No. 417,210).

PCR fragments A and B were combined in a PCR as template to obtain a 300bp fragment containing H6 linkage/Pol3/Nef2 using external primers MPSYN783/MPSYN786 (SEQ ID NOS: ). The 300bp fragment was digested with XhoI/HindIII and a 290 bp fragment was isolated and ligated with similarly digested pBSSK to generate pBS783/786. The sequence was confirmed.

(2) Pol 2/Nef central (Nef 1)/Pol 1

A 210 bp fragment (C) containing Pol2 was derived by PCR using synthetic oligonucleotides MPSYN787/MPSYN788 (SEQ ID NOS: ) and template pHXBD2.

A 270 bp fragment (D) containing Nef1 was derived by PCR using synthetic oligonucleotides MPSYN789/MPSYN790 (SEQ ID NOS: ) and template p2-60-HIV.3 (described in U.S. application Serial No. 08/417,210).

A 170 bp fragment (E) containing Pol1 was derived by PCR using primers MPSYN791/MPSYN792 (SEQ ID

NOS: ) and template pHXBD2.

Fragments C and D were combined as template in a PCR for Pol 2/Nef 1 using external primers

MPSYN787/MPSYN790 (SEQ ID NOS: ) resulting in a 460 bp  
5 PCR product (C+D).

Fragments D and E were combined as template in a PCR for Nef 1/Pol 1 using external primers

MPSYN789/MPSYN792 (SEQ ID NOS: ), resulting in isolation of a 420 bp fragment (D+E).

10 Fragments (C+D) and (D+E) were combined as template in a PCR with external primers MPSYN787/MPSYN792 (SEQ ID NOS: ) to obtain a 610 bp fragment containing Pol 2/Nef 1/Pol 1. This 610 bp fragment was digested with *HindIII*/*PstI*. The resulting 590 bp fragment was  
15 ligated with pBSSK cut with *HindIII*/*PstI* to generate pBS787/792. The sequence was confirmed.

**MPSYN783:** 5' CCC CTC GAG TCG CGA TAT CCG TTA AGT TTG TAT CGT AAT GCC ACT AAC AGA AGA AGC A 3' (58mer)

**MPSYN784:** 5' AAA TCT CCA CTC CAT CCT TGT TTT CAG ATT TTT  
20 AAA 3' (36 mer)

**MPSYN785:** 5' AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT 3' (36 mer)

**MPSYN786:** 5' CCC AAG CTT ACA ATT TTT AAA ATA TTC AGG 3' (30 mer)

25 **MPSYN787:** 5' CCC AAG CTT ATG GCA ATA TTC CAA AGT AGC 3' (30 mer)

**MPSYN788:** 5' TGG AAA ACC TAC CAT GGT TGT AAG TCC CCA CCT CAA 3' (36 mer)

**MPSYN789:** 5' TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA  
30 ACA 3' (36 mer)

**MPSYN790:** 5' TAC AGT CTC AAT CAT TGG TAC TAG CTT GTA GCA CCA 3' (36 mer)

**MPSYN791:** 5' TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA 3' (36 mer)

35 **MPSYN792:** 5' CCC CCT GCA GAA AAA TTA AGG CCC AAT TTT TGA AAT 3' (36 mer)

(SEQ ID NOS: )

Assembly of entire cassette:

A 590 bp *HindIII*/*PstI* fragment was isolated from pBS787/792 and ligated with vector pBS783/786 cut with *HindIII*/*PstI* to generate pBS783/792. pBS783/792 was  
5 cut with *EcoRV* and *PstI*, to generate an 880 bp fragment which was then ligated with similarly digested vector pBSH6-1 to generate pBSH6PN. Plasmid pBSH6PN was digested with *BamHI* and a 1060 bp fragment was isolated. pVQC5LSP1, a generic C5 donor plasmid, was digested with  
10 *BamHI* and ligated with the 1060 bp fragment from pBSH6PN. The resulting plasmid, pMPC5H6PN, contains the HIV pol/nef "string of beads" cassette in the ALVAC C5 locus.

Example 3 - Expression studies15 Example 3.1 - NYVAC Expression Results

Dishes containing confluent monolayers of cells were infected at a multiplicity of infection (moi) of 2. After incubation for specified time periods, cells were incubated in labeling medium for 1 hour. At the end of  
20 the incubation, cells were harvested for immunoprecipitation analysis as described (Harlow, E and Lane, D (1988) ; Langone, J. (1982)).

Cells were infected at an moi of 2 pfu/cell and incubated for specified time periods. At the appropriate  
25 time post-infection, cell lysates were prepared for RNA analysis. The medium was aspirated and cells were harvested. RNA was isolated and prepared using the TRI-Reagent (Molecular Research Center Inc. Cincinnati, OH. 45212) as per manufacture instructions and analyzed by  
30 slot blot. Radiolabelled DNA probes were used to detect specific RNA species.

The effect of vP1379 and vP1380 compared to the parental virus vP994 on the expression of HIV env truncated MN strain was studied by radiolabeling at  
35 specific times post-infection on CEF cells. IP analysis with monoclonal antibody against HIV env truncated MN strain (mAb K3A) revealed a significant increase in de

5    *de novo* synthesis for vP1380 infected cells at early times post infection compared to either vP994 parental virus or vP1379. A similar trend is observed at late times post infection. IP analysis with rabbit anti H4L antiserum (provided by Dr. S. Shuman, Sloan-Kettering Institute, NY) show that only vP1380 infected cells expressed H4L product early in infection. Neither vP994 nor vP1379 infected cells expressed H4L early in infection. All samples show *de novo* synthesis of H4L late in infection, 10 but expression rates are higher for vP1380 infected cells than for either vP994 or vP1379 infected cells. IP analysis of E3L product, a constitutive vaccinia protein, show that *de novo* synthesis occurs at a higher rate at all times post infection in vP1380 infected cells than in 15 either vP994 or vP1379 infected cells.

These results indicate that vP1379 is a defective recombinant with a pattern of expression identical to the parental virus unlike vP1380 recombinant which expresses H4L at early and late times post- 20 infection. This early H4 expression clearly correlates with the enhanced expression of the proteins under study (HIV *env* and E3L) at early times post-infection.

The following studies were conducted with vP1380 and vP994 since vP1379 does not express H4L product at early times post-infection. The rate of 25 expression at different times post infection in HeLa cells (non permissive system) was studied by IP analysis. IP analysis with anti-H4L shows that vP1380 infected cells expressed H4L product at all times post-infection 30 (3, 6, 24 and 48 Hrs.). No product was detected in vP994 infected cells at any time post infection. Sustained *de novo* synthesis is observed that increases with time. Analysis of HIV Env product shows that, although product expression levels are higher at all times for vP1380 35 infected cells vs. vP994's, the most significant difference is seen at late times, 24 and 48 Hrs., suggesting that expression of H4L must have an impact at



some level on expression of HIV Env product. Expression of E3L product is also increased in vP1380 infected cells compared to vP994.

Experiments performed on L929 cells gave similar results. The most significant difference was that expression rates of the H4L product at all times post infection was very low, however there was a dramatic difference in the *de novo* synthesis rate of HIV Env component. Differences in the rates of Env synthesis peaked at 24 hours with a 5 to 10 fold increase in vP1380 infected cells compared to vP994.

Since H4L product is an early transcription factor, it is of interest to determine if the results obtained at the expression level correlate with an increase in H4L message in vP1380 infected cells. RNA analysis by slot blots indicate that H4L message is detectable at all times post infection in vP1380 infected cells and achieved a steady state at 6 Hrs. post infection.

HIV Env message levels increase rapidly to steady state levels at 3 Hrs post infection and remained at those levels for all time points in vP1380 infected cells. On the other hand, vP994 infected cells show a peak of HIV env message at 6 hours post infection and a decline starting at 12 hours. E3L message in vP1380 infected cells is present at higher levels for all times post infection compared to vP994 infected cells. This pattern of RNA levels is consistent with the pattern of *de novo* synthesis rate at the protein level.

Mice were immunized by the intraperitoneal route on day 0 and 28. Starting prior to the first immunization and at two week intervals following the immunization, mice were bled from the retroorbital plexus. Sera were prepared from the collected blood by standard clotting techniques and stored frozen at -20°C until use in kinetics ELISA for antibodies reactive to the HIV

envelope glycoprotein.

High doses of vP994 or vP1380 elicited similar levels of antibodies (Table below). However, at the lowest dose,  $5 \times 10^6$  pfu, only vP1380 was capable of  
5 generating HIV antibodies. Moreover, the level of antibodies induced by the low dose was comparable to the levels of antibodies elicited by the highest dose,  $5 \times 10^7$  pfu.

At a dose too low for vP994, lacking vaccinia  
10 H4 but identical in all other respects to vP1380, to elicit an antibody response, vP1380 induced antibody responses equivalent to those elicited by the highest doses tested. Thus, the overexpression of vaccinia H4L in NYVAC may result in increased potency for inducing  
15 humoral responses.

TABLE: Antibody responses to recombinant HIV-1 MN/BRU gp160.

| 5  | KINETICS (mOD/min) |      |       |   |    |    |    |    |    |       |
|----|--------------------|------|-------|---|----|----|----|----|----|-------|
|    | WEEKS              |      |       |   |    |    |    |    |    |       |
| 10 | VIRUS              | DOSE | MOUSE | 0 | 2  | 4  | 6  | 8  | 10 | 12 14 |
| 15 | NYVAC              | HI   | a     | 0 | 1  | 0  | 1  | 2  | 2  | 1 2   |
|    |                    |      | b     | 0 | 0  | 0  | 2  | 2  | 2  | 2 2   |
|    |                    |      | c     | 0 | 0  | 0  | 2  | 1  | 1  | 1 1   |
| 20 | VP994              | HI   | a     | 0 | 6  | 8  | 42 | 45 | 44 | 44    |
|    | 45                 |      | b     | 0 | 1  | 1  | 34 | 42 | 35 | 24    |
|    | 23                 |      | c     | 3 | 1  | 3  | 34 | 40 | 31 | 33    |
| 25 | 34                 |      |       |   |    |    |    |    |    |       |
|    | VP994              | LO   | a     | 0 | 1  | 2  | 4  | 3  | 3  | 5 6   |
|    |                    |      | b     | 1 | 1  | 0  | 2  | 2  | 3  | 3 3   |
|    |                    |      | c     | 1 | 0  | 1  | 14 | 16 | 17 | 12    |
| 30 | 13                 |      |       |   |    |    |    | ✓  |    |       |
|    | VP1380             | HI   | a     | 2 | 8  | 39 | 41 | 49 | 47 | 52    |
|    | 50                 |      | b     | 3 | 12 | 45 | 49 | 46 | 51 | 54    |
| 35 | 49                 |      | c     | 1 | 7  | 35 | 42 | 41 | 43 | 40    |
|    | 39                 |      |       |   |    |    |    |    |    |       |
|    | VP1380             | LO   | a     | 1 | 2  | 3  | 49 | 45 | 47 | 46    |
| 40 | 44                 |      | b     | 0 | 1  | 2  | 30 | 30 | 34 | 36    |
|    | 40                 |      | c     | 0 | 3  | 14 | 54 | 48 | 51 | 51    |
|    | 54                 |      |       |   |    |    |    |    |    |       |

45

Mice were inoculated during weeks 0 and 4.

VP994, HIV 1 MN gp140, noncleavable, secreted envelope glycoprotein.

VP1380, HIV 1 MN gp140 + vaccinia H4L transcription factor.

VCP125, HIV 1 MN gp160.

HI dose,  $5 \times 10^7$  pfu.

LO dose,  $5 \times 10^6$  pfu.

50

As discussed above, possibly, part of the enhanced levels in vP1380 are due to enhanced transcription and expression of viral specific products such as E3L, such that there is enhanced transcription and translation involved in expression in vP1380. There was more expression of the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and more persistent levels of expression. Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994.

Another observation is that enhancement profiles are seen in restrictive early cells in the abortive early ALVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication, e.g., VERO or CEF, suggesting that the factor and the foreign DNA preferably should be expressed substantially co-temporally or contemporaneously, i.e., that preferably there should be co-expression at substantially the same time or stage, and that the time of expression, e.g., early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early, abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor early; in an abortive late system, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).

### 25 Example 3.2 - ALVAC Expression Results

#### ALVAC-HIV Recombinants

Immunoprecipitation (IP) was used to provide a semi-quantitative comparison of the temporal expression of the HIV-I cassette contained in the ALVAC recombinants in MRC-5 infected cells. Heat inactivated sera from HIV patients was obtained and used for the IP as described in the methods. The antiserum will precipitate the 120 KDa env protein and the various cleavage products from the gag protein precursor. In the analysis of the IP data it is apparent that the ALVAC recombinants vCP1431A and vCP1437A containing the E3L/K3L cassette had a significant increase in the level of expression at all

times post infection when compared to the ALVAC recombinant vCP205 without the E3L/K3L cassette.

Interestingly vCP1431A and vCP1437A had similar expression profiles; insertion of H6/H4L into an ALVAC E3L/K3L background did not enhance expression above E3L/K3L, suggesting that vaccinia H4L is not necessarily functional in ALVAC; but, manipulation of ALVAC transcriptional factors would lead to enhanced expression. Although there are homologs of vaccinia transcriptional factors in canarypox, the requirements in canarypox may be biochemically different; but, these differences can be ascertained by the skilled artisan without undue experimentation from this disclosure and the knowledge in the art. Furthermore, the present invention provides *in vitro* systems for transcriptional analysis in canarypox or fowlpox using vaccinia virus.

RNA slot blots were used to evaluate temporal transcriptional expression in MRC-5 cells infected with the ALVAC recombinants vCP205 and vCP1431A and vCP1437A. In this analysis comparisons were made to the levels of mRNA transcribed from the HIV-I cassette encoding the env and gag proteins. ALVAC recombinants containing the E3L/K3L cassette (vCP1431A and vCP1437A) did not exhibit a significant increase in the level of mRNA for the env and gag genes above that of the ALVAC recombinant vCP205.

The previously discussed role E3L/K3L plays in the down regulation of PKR in vaccinia infected cells thereby modulating translation seems to be operative in the ALVAC recombinants containing the vaccinia E3L/K3L functions. The data has shown that translation is significantly enhanced in cells infected with ALVAC recombinants containing the E3L/K3L genes, while no significant increase in the level of transcription has been detected. This exemplifies the impact of E3L/K3L expression on translation efficiency in poxvirus infected cells.

Immunoprecipitation analyses were also

performed using radiolabeled lysates derived from CEF cells infected with ALVAC parental virus, ALVAC-MN120TMG (vCP205), ALVAC-MN120TMGNPst (vCP1433), vCP1452 and vCP300, as described previously (Taylor et al., 1990),  
5 with human serum derived from HIV-seropositive individuals (anti-HIV). The analysis confirmed the expression of the envelope sequences with a molecular weight of 120kDa and the Gag precursor protein with a molecular weight of 55kDa in the recombinants but not in  
10 the parental virus. However, vCP300 exhibits diminished expression in comparison to vCP1452, i.e., vCP1452 surprisingly demonstrates enhanced expression due to expression of transcription and/or translation factors, in accordance with the invention.

15 FAC scan analysis with the Human anti-HIV antibody demonstrated expression of gp120 on the surface of HeLa cells infected with ALVAC-MN120TMGNPst (vCP1433). No fluorescence was detected on cells infected with ALVAC parental virus.

20 Appropriate expression of the inserted HIV genes was further confirmed by immunoprecipitation analysis (using polyclonal serum pool from HIV infected individuals) performed on a radiolabelled lysate of MRC5 cells infected with vCP1433 or vCP1452. The analysis  
25 confirmed the expression of the envelope sequences with a molecular weight of 120KDa and the Gag precursor protein with a molecular weight of 55 KDa in vCP1452.

vCP1452 had enhanced expression on human cells in comparison to vCP1433 and vCP300. Indeed, enhanced  
30 expression was observed with the E3L/K3L translational factor in human and canine cells.

Preliminary immunogenicity studies in mice showed no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no  
35 observed enhanced expression in murine cells.

Furthermore, in murine cells, the limiting factor of ALVAC expression is at the transcription level.

Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be an important factor in *in vitro* or *in vivo*

5 applications of the invention (note H4 data above), as may be the nature of the vector, e.g., the phenotype of the vector (e.g., abortive, and when abortive such as abortive early, abortive late); but, appropriate selection of a cell and vector phenotype and of time of  
10 expression of factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and the knowledge in the art, without undue experimentation.

#### ALVAC-FHV gB Recombinants

15 Analysis of the expression for vCP1459, vCP1460 and vCP1464 was accomplished by immunoprecipitation analysis using a sheep anti-FHV gB polyclonal sera. Human MRC-5 cells were inoculated at an moi =5 at time 0, and then pulsed for 1 hour with <sup>35</sup>S labelled methionine at  
20 times 3, 6, 24, 48 and 72 h p.i. The precipitated protein was separated on SDS-PAGE gels. Autoradiographs of these IPs were scanned using a densitometer. The methods used provide a semi-quantitative analysis of FHV gB expression at the specific time points.

25 Results show that all recombinants express the proper sized full-length, glycosylated FHV gB polypeptide (apparent MW of approximately 115 kDa). However, recombinants vCP1460 and vCP1464 show significant increase in the amount of gB protein (about 5 times)  
30 compared to vCP1459. In addition, these expression levels persist even at 72 hr p.i. Thus, it appears that the expression of vaccinia E3L/K3L in ALVAC has a significant effect on the level and persistence of FHV gB expression.

#### 35 Example 4 - Additional Vectors

Using the documents cited herein and the teaching herein, including in the foregoing Examples,

plasmid and naked DNA vectors, and additional viral vectors, including poxvirus, e.g., NYVAC, TROVAC, ALVAC, MVA, ts (temperature sensitive) mutants, or early (DNA) and late defective mutants, adenovirus, e.g., CAV such as  
5 CAV2, herpesvirus, e.g., Epstein Barr, are generated with enhanced transcription or translation or transcription and translation, e.g., by using H4L, vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) E3L, K3L,  
10 VAI, EBER, sigma 3, TRBP, or combinations thereof to modify the vector to contain at least one transcritpion factor or at least one translation factor or at least one transcription factor and at least one translation factor; and accordingly, enhanced expression, of exogenous coding  
15 nucleic acid molecules (such exogenous coding nucleic acid molecules including from documents cited herein or as otherwise known in the art, or from applying those teachings in conjunction with teachings herein) is obtained.

20           Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent  
25 variations thereof are possible without departing from the spirit or scope of the present invention.



References

Ahn, B-Y. and Moss, B. 1992. RNA polymerase-associated transcription specificity factor encoded by vaccinia virus. Proc. Natl. Acad. Sci. 89: 3536-3540.

5

Beattie, E., Denzler, K., Tartaglia, J., Paoletti, E. and Jacobs, B. L. 1995. Reversal of the interferon-sensitive phenotype of and E3L-minus vaccinia virus by expression of the reovirus S4 gene. J. Virol. 69: 499-505 ("Beattie et al. 1995a").

10

Beattie, E., Paoletti, E., and Tartaglia, J. 1995. Distinct Patterns of IFN Sensitivity Observed in Cells Infected with Vaccinia K3L<sup>-</sup> and E3L<sup>-</sup> Mutant Viruses.

15

Virology 210:254-263 ("Beattie et al 1995b")

Beattie, E., Tartaglia, J. and Paoletti, E. 1991. Vaccinia virus-encoded eIF-2a homologue abrogates the antiviral effect of interferon. Virology 183: 419-422.

20

Carroll, K., Elroy Stein, O., Moss, B. and Jagus, R. 1993. Recombinant vaccinia virus K3L gene product prevents activation of double-stranded RNA-dependent, initiation factor 2 alpha-specific protein kinase. J.

25

Biol. Chem. 268: 12837-12842.

Chang, H-W., Watson, J. and Jacobs, B. L. 1992. The vaccinia virus E3L gene encodes a double-stranded RNA-binding protein with inhibitory activity for the interferon-induced protein kinase. Proc. Natl. Acad. Sci. USA 89: 4825-4829.

30

Clark, P. A., Schwemmle, M., Schickinger, J., Hilse, K., and Clemens, M. J. 1991. Binding of Epstein-Barr virus small RNA EBER-1 to double-stranded RNA-activated protein kinase DAI. Nucleic Acids Res. 19:243-248.

35

- Davies, M. V., Chang, H. W. , Jacobs, B. L. and Kaufman, R. J. 1993. The E3L and K3L vaccinia virus gene products stimulate translation through inhibition of the double-stranded RNA-dependent protein kinase by different mechanisms. J. Virol. 67: 1688-1692.
- Davies, M. V., Elroy Stein, O., Jagus, R., Moss, B. and Kaufman, R. J. 1992. The vaccinia K3L gene product potentiates translation by inhibiting double-stranded-RNA-activated protein kinase and phosphorylation of the alpha subunit of eukaryotic initiation factor 2. J. Virol. 66: 1943-1950.
- Goebel, S. J., Johnson, G. P., Perkus, M. E., Davis, S. W., Winslow, J. P. and Paoletti, E. 1990. The complete DNA sequence of vaccinia virus. Virology 179: 247-266.
- Harlow, E. and Lane, D. (1988). Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory. 421-470.
- Hattori, M., and Sakaki, Y. (1986). Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. 152, 232-237.
- Imani, F. and Jacobs, B. L. 1988. Inhibitory activity for the interferon induced protein kinase is associated with the reovirus serotype 1 s3 protein. Proc. Natl. Acad. Sci. USA 85: 7887-7891.
- Jacobs, B. L. and Langland, J. O. 1996. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 219: 339-349.
- Langone, J. (1982). Applications of immobilized protein A in immunochemical techniques. J. Immunol. Methods. 55. 277-296.

Mathews, M. B. and Shenk, T. 1991. Adenovirus virus-associated RNA and translation control. *J. Virol.* 65: 5657-5662.

- 5 Moss, B. 1990. Regulation of vaccinia virus transcription. *Annu. Rev. Biochem.* 59: 661-688.

Moss, B. 1992. Molecular biology of poxviruses. In *Recombinant Poxviruses*. Binns M. M., Smith, G. L. (eds).

- 10 Boca Raton, FL: CRC Press; pg. 45-80.

Park, H., Davies, M. V., Langland, L. O., Chang, H-W., Nam, Y. S., Tartaglia, J., Paoletti, E., Jacobs, B. L., Kaufman, R. J. and Venkatesan, S. 1994. A cellular

- 15 protein that binds several structured viral RNAs is an inhibitor of the interferon induced PKR protein kinase... in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 91: 4713-4717.

- 20 Perkus, M., Limbach, K., Paoletti, E. (1989). Cloning and expression of foreign genes in vaccinia virus, using a host range selection system. *J. Virology* 63. 3829-3836.

Perkus, M. E., Tartaglia, J., and Paoletti, E. 1995.

- 25 Poxvirus-based vaccine candidates for cancer, AIDS and other infectious diseases. *J. of Leukocyte Biology* 58: 1-13.

Sharp, T. V. , Schwemmle, M., Jeffrey, I., Laing, K.,

- 30 Mellor, H., Proud, C. G., Hilse, K. and Clemens, M. J. 1993. Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein-Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. *Nucleic Acids Res.* 21: 4483-4490.

35

Tabor, S., and Richardson, C.C. (1987). DNA sequence analysis with a modified bacteriophage T7 polymerase.

Proc. Natl. Acad. Sci. USA 84, 4767-4771.

Tartaglia, J., Perkus, M. E., Taylor, J. et al. 1992.  
NYVAC: A highly attenuated strain of vaccinia virus.

5 Virology 188: 217-32.

Thimmappaya, B. C., Weinberger, C., Schneider, R. J. and  
Shenk, T. 1982. Adenovirus VAI RNA is required for  
efficient translation of viral mRNAs at late times after  
10 infection. Cell 31: 543-551.

Watson, J., Chang, H-W. and Jacobs, B. L. 1991.  
Characterization of a vaccinia virus-induced dsRNA-  
binding protein that may be the inhibitor of the dsRNA-  
15 dependent protein kinase. Virology 185: 206-216.

Yuen, L, and Moss, B. (1987). Oligonucleotide sequence  
signaling transcriptional termination of vaccinia virus  
early genes. Proc. Natl. Acad. Sci. USA 84, 6417-6421.

20

Zhang, Y., Ahn, B-Y. and Moss, B. 1994. Targeting of a  
multicomponent transcription apparatus into assembling  
vaccinia virus particles requires RAP94, an RNA  
polymerase-associated protein. J. Virol. 68: 1360-1370.

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WHAT IS CLAIMED IS:

1. A vector for enhanced expression of at least one first nucleic acid molecule in a cell having a particular phenotype, said vector modified to comprise  
5 the first nucleic acid molecule and at least one second nucleic acid molecule encoding a transcription factor or a transcription factor and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to  
10 the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing transcription or transcription and translation.

2. The vector of claim 1 wherein the first  
15 nucleic acid molecule is operably linked to a first promoter and the second nucleic acid molecule is operably linked to a second promoter, and the first and second promoters are functional substantially co-temporally.

3. The vector of claim 2 wherein the first  
20 and second nucleic acid molecules are at different loci within the vector.

4. The vector of claim 2 wherein the first and second nucleic acid molecules are at the same locus within the vector.

25 5. The vector of claim 1 wherein the first nucleic acid molecule and the second nucleic acid molecule are operably linked to a promoter.

6. The vector of claim 1 wherein transcription factor is of poxvirus origin.

30 7. The vector of claim 6 wherein the transcription factor is from a vaccinia virus.

8. The vector of claim 7 wherein the transcription factor is from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof.  
35

9. The vector of claim 1 wherein the second nucleic acid molecule is comprised of at least one

transcription factor and at least one translation factor.

10. The vector of claim 1 wherein the translation factor effects inhibition of eIF-2 $\alpha$  phosphorylation or inhibition of PKR phosphorylation or otherwise sequesters dsRNA, increasing the effective concentration of dsRNA.

11. The vector of claim 10 wherein said at least one second molecule is selected from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA frame, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

12. The vector of claim 1 wherein said first nucleic acid molecule encodes a molecule selected from the group consisting of an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene and a fusion protein.

13. The vector of claim 1 which is a recombinant virus.

14. The vector of claim 13 which is a recombinant poxvirus.

15. A method for preparing a vector as claimed in claim 1 comprising modifying the vector to comprise the at least one second nucleic acid molecule, and optionally also modifying the vector to comprise the first nucleic acid molecule, so that there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell.

16. The method for claim 15 comprising operably linking the first nucleic acid molecule to a first promoter and the second nucleic acid molecule to a second promoter, wherein the first and second promoters are functional substantially co-temporally.

17. The method for claim 15 comprising operably linking the first and second nucleic acid

molecules to a promoter.

18. An immunological, vaccine or therapeutic composition comprising the vector of claim 1 and a pharmaceutically acceptable carrier or diluent.

5 19. A method for generating an immunological or therapeutic response in a host comprising administering to the host the composition of claim 18.

20. A method for increasing expression of at least one first nucleic acid molecule by a vector  
10 comprising the first nucleic acid molecule, wherein the expression is in a cell having a particular phenotype, and the method comprising modifying the vector to comprise at least one second nucleic acid molecule encoding a transcription factor or a transcription factor  
15 and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by  
20 enhancing transcription or transcription and translation.

21. A method for expressing a gene product in vitro comprising infecting, or transfecting, a suitable cell with a vector as claimed in claim 1.

22. A method for expressing the first nucleic acid molecule in vivo comprising administering the vector  
25 of claim 1 to a host.

23. vCP1452 or vCP1433.

## FIG. 1

Fig. 1

Nucleotide sequence of the insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter.

| Characteristic   | Position(s)               |
|------------------|---------------------------|
| Left arm         | 1-798                     |
| Right arm        | 6636-7319                 |
| H6 promoter      | C3307-3184 and C6495-6372 |
| H4L orf          | C3183-799                 |
| T to C mutations | C2836 and C2839           |
| lacZ orf         | C6371-3327                |

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1  GGATCCTGCC GTTCCTATTCT TAGACCAAAA ATTCGGTTTTC ATGTTTTTCGA AGCGGTGTTTC
61  TGCAACAAGT CCGGGATCGT GTTCTACATA TTTGGCGGCA TTATCCAGTA TCTGCCTATT
121 GATCITTCATT TCGTTTTTCGA TTCTGGCTAT TTCAAATAAA AATCCCGATG ATAGACCTCC
181 AGACTTTTATA ATTTTCATCTA CGATGTTTCAG CGCCGTAGTA ACTCTAATAA TATAGGCTGA
241 TAAGCTAACA TCATACCCTC CTGTATATGT GAATATGGTA TGATTTTTTGT CCATTACAAG
301 CTCGGTTTTTA ACTTTATTGC CIGTAATAAT TTCTCTCATC TGTAGGATAT CTATTTTTTTT
361 GTCATGCATT GCCTTCAAGA CCGGACGAAG AAACGTAATA TCCTCAATAA CGTTATCGTT
421 TTCTACAATA ACTACATATT CTACCTTTTT ATTTTCTAAC TCGGTAAAAA AATTAGAATC
481 CCATAGGGCT AAATGTCTAG CGATATTTCT TTTTCGTTTCC TCTGTACACA TAGTGTTACA
541 AAACCCTGAA AAGAAGTGAG TATACTTGTC ATCATTCTTA ATGTTTTCTC CAGTCCACTG
601 TATAAACGCA TAATCCTTGT AATGATCTGG ATCATCCTTG ACTACCACAA CATTTCTTTT
661 TTCTGGCATA ACTTCGTGTG CTTTACATC ATCGAATCTC TGATCATTAA TATGCTCATG
721 AACATTAGGA AATGTTTTCTG ATGGAGGTCT ATCAATAACT GGCACAACAA TAACAGGAGT
781 TTTACCGGCC GCCATTTAGT TATTGAAATT AATCATATAC AACTCTTTAA TACGAGTTAT
841 ATTTTTCGTCT ATCCATTGTT TCACATTTAC ATATTTTCGAC AAAAAGATAT AAAATGCGTA
901 TTCCAATGCT TCTCTGTTTA ATGAATTACT AAAATATACA AACACGTCAC TGTCTGGCAA
961 TAAATGATAT CTTAGAATAT TGTAACAATT TATTTTGTAT TGCACATGTT CGTGATCTAT
1021 GAGTTCTTCT TCGAATGGCA TAGGATCTCC GAATCTGAAA ACGTATAAAT AGGAGTTAGA
1081 ATAATAATAT TTGAGAGTAT TGGTAATATA TAAACTCTTT AGCGGTATAA TTAGTTTTTTT
1141 TCTCTCAATT TCTATTTTTTA GATGTGATGG AAAAATGACT AATTTTGTAG CATTAGTATC
1201 ATGAATCTTA ATCAAAATCT TAATATCTTC GTCACACGTT AGCTCTTTGA AGTTTTTAAG
1261 AGATGCATCA GTTGGTTCTA CAGATGGAGT AGGTGCAACA ATTTTTTGT TACACATGT
1321 ATGTACTGGA GCCATTGTTT TAACTATAAT GGTGCTTGTA TCGAAAAACT TTAATGCAGA
1381 TAGCGGAAGC TCTTCGCGGC GACTTTCTAC ATCGTAATTG GGTCTAACG CCGATCTCTG
1441 AATGGATACT AGTTTTCTAA GTTCTAATGT GATTCTCTGA AAATGTAAAT CCAATTCCTC
1501 CGGCATTATA GATGTGTATA CATCGGTAAA TAAACTATA GTATCCAACG ATCCCTTCTC
1561 GCAAATTTCTA GTCTTAACCA AAAAATCGTA TATAACCAG GAGATGGCGT ATTTAAGAGT
1621 GGATTCTTCT ACCGTTTTGT TCTTGGATGT CATATAGGAA ACTATAAAGT CCGCACTACT
1681 GTTAAGAATG ATTACTAACG CAACTATATA GTTCAAATTA AGCATTTTGG AAACATAAAA
1741 TAACTCTGTA GACGATACTT GACTTTTCGAA TAAGTTTGCA GACAAACGAA GAAAGAACAG
1801 ACCTCTCTTA ATTTTCAGAAG AAAACTTTTT TTCGTATTCC TGACGTCTAG AGTTTATATC
1861 AATAAGAAAG TTAAGAATTA GTCGGTTAAT GTTGTATTTT ATTACCCAAG TTTGAGATTT
1921 CATAATATTA TCAAAAGACA TGATAATATT AAAGATAAAG CGCTGACTAT GAACGAAATA
1981 GCTATATGGT TCGCTCAAAA ATATAGTCTT GTTAAACGTG GAAACGATAA CTGTATTTTT
2041 AATCACGTCA CCGGCATCTA AATTAAATAT AGGTATATTT ATTCCACACA CTCTACAATA
2101 TGCCACACCA TCTTCATAAT AAATAAATTC GTTAGCAAAA TTATTAATTT TAGTGAAATA
2161 GTTAGCGTCA ACTTTTCATAG CTTCTCTCAA TCTAATTTGA TGCTCACACG GTGCGAATTC
2221 TACTCTAACA TCCCTTTTCC ATGCCTCAGG TTCATCGATC TCTATAATAT CTAGTTTTTT
2281 GCGTTTTACA AACACAGGCT CGTCTCTCGC GATGAGATCT GTATAGTAAC TATGTAAATG
2341 ATAAGTAGAT AGAAAGATGT AGCTATATAG ATGACGATCC TTTAAGAGAG GTATAATAAC
2401 TTTACCCCAA TCAGATAGAC TGTGTGTTATG GTCTTCGGAA AAAGAATTTT TATAAATTTT

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Fig. 1 (cont'd)

|      |             |             |             |             |             |             |
|------|-------------|-------------|-------------|-------------|-------------|-------------|
| 2461 | TCCAGTATTT  | TCCAAATATA  | CGTACTTAA   | ATCTAAAAA   | TCCTTAATGA  | TAATAGGAAT  |
| 2521 | GGATAATCCG  | TCTATTTTAT  | AAAGAAATAC  | ATATCGCACA  | TTATACITTT  | TTTTGGAAAT  |
| 2581 | GGGAATACCG  | ATGTGTCTAC  | ATAAATATGC  | AAAGTCTAAA  | TATTTTTTAG  | AGAATCTTAG  |
| 2641 | TTGGTCCAAA  | TTCTTTTCCA  | AGTACGGTAA  | TAGATTTTTT  | ATATTGAACG  | GTATCTTCTT  |
| 2701 | AATCTCTGGT  | TCTAGTTCCG  | CATTAAATGA  | TGAAACTAAG  | TCACTATTTT  | TATAACTAAC  |
| 2761 | GATTACATCA  | CCTCTAACAT  | CATCAATTTAC | CAGAATACTG  | ATCTTCTTTT  | GTGTAATAA   |
| 2821 | CATGTCTAAT  | GTGTTGAAGA  | AAAGATCATA  | CAAGTTATAC  | GTCAITTCAT  | CTGTGGTATT  |
| 2881 | CTTGTGCTTG  | AAGGATAAAC  | TCGTACTAAT  | CTCTTCTTTA  | ACAGCCTGTT  | CAAAATTTATA |
| 2941 | TCCTATATAC  | GAAAAAATAG  | CAACCAGTGT  | TTGATCATCC  | GCGTCAATAT  | TCGTTCCTAT  |
| 3001 | CGTAGTGTAT  | AACAATCGTA  | TATCTTCTTC  | TGTGATAGTC  | GATACGTTAT  | AAAGGTTGAT  |
| 3061 | AACGAAAATA  | TTTTTATTTT  | GTGAAATAAA  | GTCAATCGTAG | GATTTTGGAC  | TTATATTCCG  |
| 3121 | GTCTAGTAGA  | TATGCTTTTA  | TTTTTGGAA   | GATCTCAATT  | AGAATAGTCT  | CTTTAGAGTC  |
| 3181 | CATTACGATA  | CAAACTTAAC  | GGATATCGCG  | ATAATGAAAT  | AATTTATGAT  | TATTTCTCGC  |
| 3241 | TTTCAATTTA  | ACACAACCCT  | CAAGAACCCT  | TGTATTTATT  | TTCACTTTT   | AAGTATAGAA  |
| 3301 | TAAAGAAGCT  | TCCCGGGGGA  | TCCTTATTTT  | TGACACCAGA  | CCAACCTGTA  | ATGGTAGCGA  |
| 3361 | CTTGTGCTTG  | CGCGGAATC   | CGCCGATCT   | GACGGGCTCC  | AGGAGTCGTC  | GCCACCAATC  |
| 3421 | CCCATATGGA  | AACCGTCGAT  | ATTACGCCAT  | GTGCCITCTT  | CCGCGTGCAG  | CAGATGGCGA  |
| 3481 | TGGCTGGTTT  | CCATCAGTTG  | CTGTTGACTG  | TAGCGGCTGA  | TGTTGAACTG  | GAAGTCGCCG  |
| 3541 | CGCCACTGGT  | GTGGGCCATA  | ATTCAAITTCG | CGCGTCCCGC  | AGCGCAGACC  | GTITTCGCTC  |
| 3601 | GGGAAGACGT  | ACGGGGTATA  | CATGTCCTGAC | AATGGCAGAT  | CCCAGCGGTC  | AAAACAGGCG  |
| 3661 | GCAGTAAGGC  | GGTGGGATA   | GTITTTCTGCG | GGCCCTAATC  | CGAGCCAGTT  | TACCGCTCT   |
| 3721 | GCTACCTGCG  | CCAGCTGGCA  | GTTTCAGGCCA | ATCCGCGCCG  | GATGCGGTGT  | ATCGCTCGCC  |
| 3781 | ACTTCAACAT  | CAACGGTAA   | CGCCATTTGA  | CCACTACCAT  | CAATCCGGTA  | GGTTTTCCGG  |
| 3841 | CTGATAAAT   | AGGTTTTCCC  | CTGATGCTGC  | CACGCGTGAG  | CGGTCTTAAT  | CAGCACCGCA  |
| 3901 | TCAGCAAGTG  | TATCTGCCGT  | GCATGCGAAC  | AACGCTGCTT  | CGGCCCTGTA  | ATGGCCCGCC  |
| 3961 | GCCTTCCAGC  | GTTTCGACCCA | GGCGTTAGGG  | TCAATGCGGG  | TGCTTCACT   | TACGCCAATG  |
| 4021 | TCGTTATCCA  | GCGGTGCACG  | GGTGAACCTGA | TCCGCGCAGCG | GCGTCAGCAG  | TGTTTTTTATA |
| 4081 | TCGCCAATCC  | ACATCTGTGA  | AAGAAAGCCT  | GACTGGCGGT  | TAAATTGCCA  | ACGCTTATTA  |
| 4141 | CCCAGCTCGA  | TGCAAAAATC  | CATTTTCGCTG | TGCGTCAGAT  | GCGGGATGGC  | GTGGGACGGC  |
| 4201 | GCGGGGAGCG  | TCACACTGAG  | GTITTTCCGCC | AGACGCCACT  | GCTGCCAGGC  | GCTGATGTGC  |
| 4261 | CCGGCTTCTG  | ACCATGCGGT  | CGCGTTCCGGT | TGCACTACGC  | GTACTGTGAG  | CCAGAGTTGC  |
| 4321 | CCGGCGCTCT  | CCGGCTCGCG  | TAGTTCCAGG  | AGTTCAATCA  | ACTGTTTACC  | TTGTGGAGCG  |
| 4381 | ACATGCTGAG  | GCACCTTACC  | GCTTCCAGC   | GGCTTACCAT  | CCAGCGCCAC  | CATCCAGTGC  |
| 4441 | AGGAGCTCGT  | TATCGCTATG  | ACGGAACAGG  | TATTCGCTGG  | TCACTTCGAT  | GGTTTGCCCG  |
| 4501 | GATAAACCGA  | ACTGGAAAAA  | CTGCTGCTGG  | TGTTTTGCCT  | CCGTCAGCGC  | TGGATGCGGC  |
| 4561 | GTGCGGTCCG  | CAAAGACCAG  | ACCGTTTCATA | CAGAACTGGC  | GATCGTTCCG  | CGTATCGCCA  |
| 4621 | AAATCACCAG  | CGTAAGCCGA  | CCACGGGTTG  | CCGTTTTTCAT | CATATTTAAT  | CAGCGACTGA  |
| 4681 | TCCACCCAGT  | CCCAGACGAA  | GCCGCGCTGT  | AAACGGGGAT  | ACTGACGAAA  | CGCTGCCAG   |
| 4741 | TATTTAGCGA  | AACCGCCAAG  | ACTGTTACCC  | ATCGCGTGGG  | CGTATTCGCA  | AAGSATCAGC  |
| 4801 | GGGCGCGTCT  | CTCCAGGTAG  | CGAAAGCCAT  | TTTTTGTATG  | ACCATTTCCG  | CACAGCCGGG  |
| 4861 | AAGGGCTGGT  | CTTCAATCCAC | GCGCGCGTAC  | ATCGGGCAAA  | TAATATCGGT  | GGCCGTGGTG  |
| 4921 | TCGGCTCCCG  | CGCCTTTCATA | CTGCACCGGG  | CGGGAAGGAT  | CGACAGATTT  | GATCCAGCGA  |
| 4981 | TACAGCGCGT  | CGTGATTAGC  | GCCGTGGCCT  | GATTCATTCC  | CCAGCGACCA  | GATGATCACA  |
| 5041 | CTCGGGTGAT  | TACGATCGCG  | CTGCACCAIT  | CGCGTTACGC  | GTTCGCTCAT  | CGCCGGTAGC  |
| 5101 | CAGCGCGGAT  | CATCGGTCAG  | ACGATTCAIT  | GGCACCATGC  | CGTGGGTTTC  | AATATTGGCT  |
| 5161 | TCATCCACCA  | CATACAGGCC  | GTAGCGGTGC  | CACAGCGTGT  | ACCACAGCGG  | ATGGTTCCGA  |
| 5221 | TAATGCGAAC  | AGCGCACGGC  | GTTAAAGTTG  | TTCTGCTTCA  | TCAGCAGGAT  | ATCCTGCACC  |
| 5281 | ATCGTCTGCT  | CATCCATGAC  | CTGACCATGC  | AGAGGATGAT  | GCTCGTGACG  | GTTAACGCCCT |
| 5341 | CGAATCAGCA  | ACGGCTTGCC  | GTTTCAGCAG  | AGCAGACCAT  | TTTCAATCCG  | CACCTCCGGG  |
| 5401 | AAACCGACAT  | CGCAGGCTTC  | TGCTTCAATC  | AGCGTGCCGT  | CGGCGGTGTG  | CAGTTCAACC  |
| 5461 | ACCGCACGAT  | AGAGATTCCG  | GATTTCCGGC  | CTCCACAGTT  | TCGGGTTTTT  | GACGTTTCAGA |
| 5521 | CGTAGTGTGA  | CGCGATCGGC  | ATAACCACCA  | CGCTCATCGA  | TAATTTTACC  | GCCGAAAGGC  |
| 5581 | GCGGTGCGCG  | TGGCGACCTG  | CGTTTCACCC  | TGCCATAAAG  | AAACTGTTAC  | CCGTAGGTAG  |
| 5641 | TCACGCAACT  | CGCGCACAT   | CTGAACCTTCA | GCCTCCAGTA  | CAGCGCGGCT  | GAAATCATCA  |
| 5701 | TTAAAGCGAG  | TGGCAACATG  | GAAATCGCTG  | ATTTGTGTAG  | TCGGTTTTATG | CAGCAACCGAG |
| 5761 | ACGTACCGGA  | AAATGCCGCT  | CATCCGCCAC  | ATATCCTGAT  | CTTCCAGATA  | ACTGCCGTCA  |
| 5821 | CTCCAACGCA  | GCACCATCAC  | CGCGAGGCGG  | TTTTCTCCGG  | CGCGTAAAAA  | TGCGCTCAGG  |
| 5881 | TCAAATTTCAG | ACGCGCAACG  | ACTGTCTGTG  | CCGTAAACCGA | CCCAGCGCCC  | GTTCACCCAC  |
| 5941 | AGATGAAACG  | CCGAGTTAAC  | GCCATCAAAA  | ATAATTCGCG  | TCTGGCCTTC  | CTGTAGCCAG  |
| 6001 | CTTTCATCAA  | CATTAAATGT  | GAGCGAGTAA  | CAACCGTTCG  | GATTCTCCGT  | GGGAACAAAC  |
| 6061 | GGCGGATTGA  | CCGTAATGGG  | ATAGGTTACG  | TTGGTGTAGA  | TGGGCGCATC  | GTAACCGTGC  |
| 6121 | ATCTGCCAGT  | TTGAGGGGAC  | GACGACAGTA  | TGGCCTCAG   | GAAGATCGCA  | CTCCAGCCAG  |

## Fig. 1 (cont'd)

```
6181 CTTTCCGGCA CCGCTTCTGG TGCCGGAAAC CAGGCAAAGC GCCATTGCCC ATTCAGGCTG
6241 CGCAACTGTT GGGGAAGGGCG ATCGGTGCGG GCCTCTTCGC TATTACGCCA GCTGGCGAAA
6301 GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG GGTTTTCCCA GTCACGACGT
6361 TGTAAACCA TTACGATACA AACTTAACGG ATATCGCGAT AATGAAATAA TTTATGATTA
6421 TTTCTCGCTT TCAATTTAAC ACAACCCTCA AGAACCTTTG TATTTATTTT CACTTTTAA
6481 GTATAGAATA AAGAACC CGG GAAGCTTGTC TAGCTGGTGC TGAGTTTCTA CGTGAGTTGA
6541 TTCGTCTCTT GCGTGCCTCT CGTGATCCAA TTGTCCCGAG ATATTCTCTT CTCTCCGGT
6601 GCTTCTTATG AAACTTTCCC TGACGGTGGC GTTTTAAAGT TACAAACAAC TAGGAAATTG
6661 GTTTATGATG TATAATTTTT TTAGTTTTTA TAGATTCTTT ATTCTATACT TAAAAAATGA
6721 AAATAAATAC AAAGGTTCTT GAGGGTTGTG TTAAATTGAA AGCGAGAAAT AATCATAAAT
6781 TATTTCAATTA TCGCGATATC CGTTAAGTTT GTATCGTAAT GGCGTGGTCA ATTACAAATA
6841 AAGCGGATAC TAGTAGCTTC ACAAAGATGG CTGAAATCAG AGCTCATCTA AAAAATAGCG
6901 CTGAAATAA AGATAAAAAC GAGGATATTT TCCCGGAAGA TGTAATAATT CCATCTACTA
6961 AGCCCAAAAC CAAACGAGCC ACTACTCCTC GTAAACCAGC GGCTACTAAA AGATCAACCA
7021 AAAAGGAGGA AGTGAAGAA GAAGTAGTTA TAGAGGAATA TCATCAAACA ACTGAAAAAA
7081 ATTCTCCATC TCCTGGAGTC GGCACATTG TAGAAAGCGT GGCTGCTGTA GAGCTCGATG
7141 ATAGCGACGG GGATGATGAA CCTATGGTAC AAGTTGAAGC TGGTAAAGTA AATCATAGTG
7201 CTAGAAGCGA TCTTTCTGAC CTAAAGGTGG CTACCGACAA TATCGTTAAA GATCTTAAGA
7261 AAATTATTAC TAGAATCTCT GCAGTATCGA CGGTTCTAGA GGATGTTCAA GCAGGATCC
```

Fig. 2

Fig. 2

Nucleotide Sequence of the ALVAC C8 Insertion site containing the  
H5/H4L expression cassette

## Characteristic

## Position(s)bp

Left Arm

1-487

Right Arm

3016-4225

H6 Promoter

495-618

H4L ~~cat~~

619-3003

```

1 GAGCTCACTT ATTACATCCT ACTGACTATA TACAGCGAAT TAACCATAGG CGTAATTGTA
61 CAGAAACCAG GAAATTATTA CCGCCTTTTA TAAGAAGTAT TAATAAAACA TGTAGCGTAT
121 GTCTAGAAAG AATATACGAA AAAGAAATAA ATAAACAATA TTTCGGTATT TTACCAAATT
181 GTAAACACCGT GTTTTGTTTT TACTGTATAC AACGTTGGAT GTCTATAATA AAAGGTACGG
241 ATACCGAAGG TACATGTCCT GTATGTAGAA CAGTTTCTGT ATTTATAGTG CCTAATAGGT
301 ACTGGATAGA CGATAAATAT GAAAAGAGAT TAATTATAAA TAAATATAAG AATGACAGAA
361 AGACTTATAA AGCGTTTTAA CATTATATAG GAAGATACGT ATTATTTTAT ACAGTAAACA
421 ACAGTTTATT TGTTACTAAT GATTAAAGTA CGTGAATAAT TAGCTATAAA AAGGATCCAT
481 CGATGATGGG AAGCTTCTTT ATTCTATACT TAAAAAGTGA AAATAAATAC AAAGGTTCTT
541 GAGGGTTGTG TTAAATTGAA AGCGAGAAAT AATCATAAAT TATTTTCATTA TCGCGATATC
601 CGTTAAGTTT GTATCGTAAT GGACTCTAAA GAGACTATTC TAATTGAGAT CATTCCAAAA
661 ATAAAAGCAT ATCTACTAGA CGCGAATATA AGTCCAAAAA CCTACGATGA CTTTATTTC
721 CGAAATAAAA ATATTTTCGT TATCAACCTT TATAACGTAT CGACTATCAC AGAAGAAGAT
781 ATACGATTGT TATACACTAC GATAGAACAG AATATTGACG CGGATGATCA AACACTGGTT
841 GCTATTTTTT CGTATATAGG ATATAAATT ATATAAATT GAACAGGCTG TTAAGAAGA GATTAGTACG
901 AGTTTATCCT TCAATGACAA GAATACCACA GATGAAATGA CGTATAACTT GTATGATCTT
961 TTCTTCAACA CATTAGACAT GTATTTACGA CAAAAGAAGA TCAGTATTCT GGTAAATGAT
1021 GATGTTAGAG GTGATGTAAT CGTTAGTTAT AAAAAATAGT ACTTAGTTT ATCATTTAAT
1081 GCGGAAC TAG AACCAGAGAT TAAGAAGATA CCGTTCAATA TGAAAAATCT ATTACCGTAC
1141 TTGGAAAAGA ATTTGGACCA ACTAAGATTC TCTAAAAAAT ATTTAGACTT TGCATATTTA
1201 TGTAGACACA TCGGTATTCC CATTTCCAA AAAAAAGTATA ATGTGCGATA TGTATTTCTT
1261 TATAAAATAG ACGGATTATC CATTCTTATT ATCATTAAAG ATTTTTTAGA TGTTAAGTAC
1321 GTATATTTGG AAAATACTGG AAAAATTTAT AAAAATTCTT TTCCGAAGA CCATAACAAC
1381 AGTCTATCTG ATTGGGGTAA AGTTATTATA CCTCTCTTAA AGGATCGTCA TCTATATAGC
1441 TACATCTTTC TATCTAGTTA TCATTTACAT AGTTACTATA CAGATCTCAT CGCGAGAGAC
1501 GAGCCTGTGT TTGTGAACG CAAAAAATA GATATTATAG AGATCGATGA ACCTGAGGCA
1561 TGGAAAAGGG ATGTTAGAGT AGAATTCGCA CCGTGTGAGC ATCAAATTAG ATTGAAGGAA
1621 GCTATGAAAG TTGACGCTAA CTATTTCACT AAAATTAATA ATTTTGCTAA CGAATTTATT
1681 TATTATGAAG ATGGTGTGGC ATATTGTAGA GTGTGTGGAA TAAATATACC TATATTTAAT
1741 TTAGATGCCG CTGACGTGAT TAAAAATACA GTTATCGTTT CCACGTTTAA CAAGACTATA
1801 TTTTGGAGCG AACCATATAG CTATTTCTGT CATAGTCAGC GCTTTATCTT TAATATTATC
1861 ATGTCTTTTG ATAATATTAT GAAATCTCAA ACTTGGGTAA TGAATACAA CATTAAACGA
1921 CTAATTCTTA ACTTTCTTAT TGATATAAAC TCTAGACGTC AGGAATACGA AAAAAAGTTT
1981 TCTTCTGAAA TTAAGAGAGG TCTGTTCTTT CTTCGTTTGT CTGCAAACTT ATTCGAAAGT
2041 CAAGTATCGT CTACAGAGTT ATTTTATGTT TCCAAAATGC TTAATTTGAA CTATATAGTT
2101 GCGTTAGTAA TCATTCTTAA CAGTAGTGCG GACTTTATAG TTTCTATAT GACATCCAAG
2161 AACAAAACGG TAGAAGAATC CACTCTTAAA TACGCCATCT CCGTGGTTAT ATACGATTTT
2221 TTGGTTAAGA CTAGAATTG CGAGAAGGGA TCGTTGGATA CTATAGTTTT ATTTACCGAT
2281 GTATACACAT CTATAATGCC GGAGGAATTG GATTTACATT TTCAGAGAA CACATTAGAA
2341 CTTAGAAAAC TAGTATCCAT TCAGAGATCG GCGTTAGAAC CCAATTACGA TGTAGAAAGT
2401 CGCGGCGAAG AGCTTCCGCT ATCTGCATTA AAGTTTTTTC ATACAAGCAC CATTATAGTT
2461 AAAACAATGG CTCCAGTACA TACATGTGTA GAACAAAAAA TTGTTGCACC TACTCCATCT
2521 GTAGAACCAC CTGATGCATC TCTTAAAAAC TTCAAAGAGC TAACGTGTGA CGAAGATATT
2581 AAGATTTTGA TTAGAGTTCA TGATACTAAT GCTACAAAAT TAGTCATTTT TCCATCACAT
2641 CTA AAAATAG AAATTGAGAG AAAAAAATA ATTATACCGC TAAAGAGTTT ATATATTACC
2701 AATACTCTCA AATATTATTA TTCTAACTCC TATTTATACG TTTTCAGATT CGGAGATCCT
2761 ATGCCATTCC AAGAAGAACT CATAGATCAC GAACATGTGC AATACAAAAT AAATTGTTAC
2821 AATATTCTAA GATATCATTT ATTGCCAGAC AGTGACGTGT TTGTATATT TAGTAATTCA
2881 TTA AACAGAG AAGCATTTGA ATACGCTTTT TGTCGAAATA TGTAATGTG
2941 AAACAATGGA TAGACGAAAA TATAACTCGT ATTAAAGAGT TGTATATGAT TAATTTCAT
3001 AACTAAAAGC TTCCCATCCT GCAGCTCGAG TTTTATGAC TAGTTAATCA CGGCCGCTCA

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*Fig. 2 (cont'd)*

|      |            |            |            |            |            |             |
|------|------------|------------|------------|------------|------------|-------------|
| 3061 | ATATTGTATT | GGATGGTTAG | AGATCAAAGG | ATACAAGATA | ACTGGGCTCA | TTCAGCTTT   |
| 3121 | ACATTCATCC | CTATAAGCTT | TCATAATGGG | ATTTTCTCC  | ATAATGTCAA | AATCACTTTG  |
| 3181 | GATATATTCA | AAATTTTCTA | CAAAATGTTT | TGGTTGTTCT | GAGCTAAACA | CGATGTTAGA  |
| 3241 | TATTAATAAC | TTTGCTATCT | CAAGACCTTC | TGAAGTATCA | ACTTTGATAT | TGGAAAGAGG  |
| 3301 | TGTAAAATAA | GGTGATGAAG | CGATTGTTGT | ATCTGCACAG | AATGTTAACA | GTATATCTAC  |
| 3361 | TAATTCTACA | TTTCCATCTG | TCACAGCATG | CCATAGAGGA | GTATTCCAGT | ACCTGTCCTT  |
| 3421 | AGCATTTATA | TCAGCACCGA | ATTCCAAAAG | CATAATAGTT | ATCTTTACAG | ATCCTATACA  |
| 3481 | CACAGCATAA | TGCAAAGGAG | TCATCCTATG | GCTATCTTTA | ACGTTAGTAT | ATGCTCCAGC  |
| 3541 | TAGAAGTAAT | TGCTCTATTA | TCTCCATGTT | TTCAGATTTA | ACAGCATAAT | GCAATGGATA  |
| 3601 | CATATATCCT | CTGTAACCAT | AATTTATACT | CGATCCAGCT | TTTAGTAACA | TACTCACAAAT |
| 3661 | TTCCAAATTT | TCTCTCTTTA | TAGCCTCGAT | TATGGGATGA | TTTTCCCTGT | ACTCATTGTC  |
| 3721 | AACATCAGCG | TTATACTCCA | GAAGTAACTT | TACAATTTCC | ACATTCTCTA | TAGAGACAGC  |
| 3781 | ATACTGGAGT | GGAGTCTTTA | CTTTGTAGTC | CTCATATGTA | TCCACATTAG | CGCCATGATC  |
| 3841 | CAACAAGAGT | TTCACCAGAT | CTATGTTCTG | AACTTTGACA | GCTCTATGCA | ACGGAGAAGA  |
| 3901 | TACTTGTTCC | CTAGATATAT | CAGGATCAGC | TCCTGCTAAC | AATAGAGCTT | TGGCTATTTT  |
| 3961 | AAATTTTTC  | TTTTCTACAG | CACAATGAAG | GGGTGAGCAG | CCATAATCGT | TGAATACGTC  |
| 4021 | CAGGTTAATG | CCGGTTTTCA | CAATATCTAG | CACGCTAGAC | AGAGATCCAG | ATTCAATAGC  |
| 4081 | TTCCAATAAG | TATGCCTCCA | TTTTGTGTAA | TAGTAGTAAG | TAATAATTTT | CTGAAGAAAC  |
| 4141 | TACTAACTTA | CCGAGCTATA | GTAGATAGTT | ATAATTTTCT | TTTTTTACAA | GTAGTATCAC  |
| 4201 | ATAGTGATTG | CTTATTAAAG | GTACC      |            |            |             |

## Fig. 3

Fig. 3

Nucleotide sequence of the ALVAC C6 insertion site containing the H6 / K3L and E3L expression cassette.

| Characteristic | Position(s) |
|----------------|-------------|
| Left Arm       | 1-385       |
| Right Arm      | 3273-4434 ✓ |
| K3L orf        | C727-464    |
| H6 Promoter    | C850-728    |
| E3L            | C2758-2188  |

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1  GAGCTCGCGG  CCGCCTATCA  AAAGTCITTA  TGAGTTAGGT  GTAGATAGTA  TAGATATTAC
61  TACAAAGGTA  TTCATATTTT  CTATCAATT  TAAAGTAGAT  GATATTAATA  ACTCAAAGAT
121  GATGATAGTA  GATAATAGAT  ACGCTCATAT  AATGACTGCA  AATTTGGACG  GTTCACATTT
181  TAATCATCAC  GCGTTCATAA  GTTTCAACTG  CATAGATCAA  AATCTCACTA  AAAAGATAGC
241  CGATGTATTT  GAGAGAGATT  GGACATCTAA  CTACGCTAAA  GAAATTACAG  TTATAAATAA
301  TACATAATGG  ATTTTGTTAT  CATCAGTTAT  ATTTAACATA  AGTACAATAA  AAAGTATTAA
361  ATAAAAATAC  TTACTTACGA  AAAAATGACT  AATTAGCTAT  AAAAACCAG  ATCTCTCGAG
421  GTCGACGGTA  TCGATAAGCT  TGATATCGAA  TTCATAAAAA  TTATGATGT  CTACACATCC
481  TTTTGTAATT  GACATCTATA  TATCCTTTTG  TATAATCAAC  TCTAATCACT  TTAACCTTTA
541  CAGTTTTCCC  TACCAGTTTA  TCCCTATATT  CAACATATCT  ATCCATATGC  ATCTTAACAC
601  TCTCTGCCAA  GATAGCTTCA  GAGTGAGGAT  AGTCAAAAAG  ATAAATGTAT  AGAGCATAAT
661  CCTTCTCGTA  TACTCTGCCC  TTTATTACAT  CGCCCGCATT  GGGCAACGAA  TAACAAAATG
721  CAAGCATACG  ATACAAACTT  AACGGATATC  GCGATAATGA  AATAATTTAT  GATTATTTCT
781  CGCTTTCAAT  TTAACACAAC  CCTCAAGAAG  CTTTGTATTT  ATTTTCACTT  TTTAAGTATA
841  GAATAAAGAA  AGCTCTAATT  AATTAAATGA  CAGATTGTTT  CGTTTTCCCC  TTGGCGTATC
901  ACTAATTAAT  TAACCCGGGC  TGCAGCTCGA  GGAATTCAAC  TATATCGACA  TATTTCATTT
961  GTATACACAT  AACCATTACT  AACGTAGAAT  GTATAGGAAG  AGATGTAACG  GGAACAGGGT
1021  TTGTTGATT  GCAAACATTT  CTAATACATA  ATTCTTCTGT  TAATACGCT  TGACCGTAAT
1081  CTATTATAGA  TGCCAAAGTA  TCTATATAAT  TATTTTGTAA  GATGATGTTA  ACTATGTGAT
1141  CTATATCTAA  AGTGAATAA  TCTATGTAAT  TCGATATATG  TTCCAACCT  GTCTTTGTGA
1201  TGTCTAGTTT  CGTAATATCT  ATAGCATCCT  CAAAAAATAT  ATTTCGCAT  ATTCCCAAGT
1261  CTTCAGTTCT  ATCTTCTAAA  AAATCTTCAA  CGTATGGAAT  ATAATAATCT  ATTTTACCTC
1321  TTCTGATATC  ATTAATGATA  TAGTTTTTGA  CACTATCTTC  TGTCAATTGA  TTCTTATTCA
1381  CTATATCTAA  GAAACGGATA  GCGTCCCTAG  GACGAACCTAC  TGCCATTAA  ATCTCTATTA
1441  TAGCTTCTGG  ACATAATTCA  TCTATTATAC  CAGAATTAAT  GGGAACTATT  CCGTATCTAT
1501  CTAACATAGT  TTTAAGAAAG  TCAGAATCTA  AGACCTGATG  TTCATATATT  GGTTCATACA
1561  TGAAATGATC  TCTATTGATG  ATAGTGACTA  TTTCAATCTC  TGAAAATTGG  TAACTCAATC
1621  TATATATGCT  TTCTTTGTTG  ATGAAGGATA  GAATATACTC  AATAGAATTT  GTACCAACAA
1681  ACTGTTCTCT  TATGAATCGT  ATATCATCAT  CTGAAATAAT  CATGTAAGGC  ATACATTTAA
1741  CAATTAGAGA  CTTGTCTCCT  GTTATCAATA  TACTAATCTT  GTGATAATTT  ATGTGTGAGG
1801  CAAATTTGTC  CACGTTCTTT  AATTTTGTTA  TAGTAGATAT  CAAATCCAAT  GGAGCTACAG
1861  TTCTTGGCTT  AAACAGATAT  AGTTTTCTCG  GAACAAATTC  TACAACATTA  TTATAAAGGA
1921  CTTTGGGTAG  ATAAGTGGGA  TGAAATCCTA  TTTTAATTAA  TGCTATCGCA  TTGTCCTCGT
1981  GCAAATATCC  AAACGCTTTT  GTGATAGTAT  GGCATTCAAT  GTCTAGAAAC  GCTCTACGAA
2041  TATCTGTGAC  AGATATCATC  TTTAGAGAAT  ATACTAGTCG  CGTTAATAGT  ACTACAATTT
2101  GTATTTTTTA  ATCTATCTCA  ATAAAAAAT  TAATATGTAT  GATTCAATGT  ATAACATAAC
2161  TACTAATGTT  TATTGATAAC  TAGAATCAGA  ATCTAATGAT  GACGTAACCA  AGAAGTTTAT
2221  CTAATGCTCA  TTTAGCTGCA  TTATTTTATG  CATCTCGTTT  AGATTTTCCA  TCTGCCTTAT
2281  CGAATACTCT  TCCGTCGATG  TCTACACAG  CATAAAATGT  AGGAGAGTTA  CTAGGCCCAA
2341  CTGATTTCAAT  ACGAAAAGAC  CAATCTCTCT  TAGTTATTTG  GCAGTACTCA  TTAATAATGG
2401  TGACAGGGTT  AGCATCTTTC  CAATCAATAA  TTTTTTTAGC  CGGAATAACA  TCATCAAAGG
2461  ACTTATGATC  CTCTCTCATT  GATTTTTTCG  GGGATACATC  ATCTATTATG  ACGTCAGCCA

```

## Fig. 3 (cont'd)

|      |             |             |             |             |             |             |
|------|-------------|-------------|-------------|-------------|-------------|-------------|
| 2521 | TAGCATCAGC  | ATCCGGCTTA  | TCCGCCTCCG  | TTGTCATAAA  | CCAACGAGGA  | GGAATATCGT  |
| 2581 | CGGAGCTGTA  | CACCATAGCA  | CTACGTTGAA  | GATCGTACAG  | AGCTTTATTA  | ACTTCTCGCT  |
| 2641 | TCTCCATATT  | AAGTTGTCTA  | GTTAGTTGTG  | CAGCAGTAGC  | TCCTTCGATT  | CCAATGTTTT  |
| 2701 | TAATAGCCGC  | ACACACAATC  | TCTGCGTCAG  | AACGCTCGTC  | AATATAGATC  | TTAGACATTT  |
| 2761 | TTAGAGAGAA  | CTAACACAAC  | CAGCAATAAA  | ACTGAACCTA  | CTTTATCATT  | TTTTTATTCA  |
| 2821 | TCATCCTCTG  | GTGGTTCGTC  | GTTTTCTATCG | AATGTAGCTC  | TGATTAACCC  | GTCATCTATA  |
| 2881 | GGTGTATGCTG | GTTCTGGAGA  | TTCTGGAGGA  | GATGGATTAT  | TATCTGGAAG  | AATCTCTGTT  |
| 2941 | ATTTCCCTTGT | TTTCATGTAT  | CGATTGCGTT  | GTAACATTAA  | GATTGCGAAA  | TGCTCTAAAT  |
| 3001 | TTGGGAGGCT  | TAAAGTGTG   | TTTGCAATCT  | CTACACGCGT  | GTCTAACTAG  | TGGAGGTTTCG |
| 3061 | TCAGCTGCTC  | TAGTTTGAAT  | CATCATCGGC  | GTAGTATTCC  | TACTTTTACA  | GTTAGGACAC  |
| 3121 | GGTGTATTGT  | ATTTCTCGTC  | GAGAACGTTA  | AAATAATCGT  | TGTAACCTAC  | ATCCTTTATT  |
| 3181 | TTATCTATAT  | TGTATTCTAC  | TCCTTTCCTA  | ATGCATTTTA  | TACCGAATAA  | GAGATAGCGA  |
| 3241 | AGGAATTCCT  | TTTATTGATT  | AACTAGTCAA  | ATGAGTATAT  | ATAATTGAAA  | AAGTAAAATA  |
| 3301 | TAAATCATAT  | AATAATGAAA  | CGAAATATCA  | GTAATAGACA  | GGAACTGGCA  | GATTCTTCTT  |
| 3361 | CTAATGAAGT  | AAGTACTGCT  | AAATCTCCAA  | AATTAGATAA  | AAATGATACA  | GCAAATACAG  |
| 3421 | CTTCATTCAA  | CGAATTACCT  | TTTAATTTTT  | TCAGACACAC  | CTTATTACAA  | ACTAACTAAG  |
| 3481 | TCAGATGATG  | AGAAAAGTAAA | TATAAAATTTA | ACTTATGGGT  | ATAATATAAT  | AAAGATTTCAT |
| 3541 | GATATTAAATA | ATTTACTTAA  | CGATGTTAAT  | AGACTTATTC  | CATCAACCCC  | TTCAAACCTT  |
| 3601 | TCTGGATATT  | ATAAAATACC  | AGTTAATGAT  | ATTAAAATAG  | ATTGTTTAAAG | AGATGTAAAT  |
| 3661 | AATTATTATG  | AGGTAAAGGA  | TATAAAATTA  | GTCTATCTTT  | CACATGGAAA  | TGAATTACCT  |
| 3721 | AATATTAAATA | ATTATGATAG  | GAATTTTTTA  | GGATTTACAG  | CTGTTATATG  | TATCAACAAT  |
| 3781 | ACAGGCAGAT  | CTATGGTTAT  | GGTAAAACAC  | TGTAACGGGA  | AGCAGCATTTC | TATGGTAACT  |
| 3841 | GGCCTATGTT  | TAATAGCCAG  | ATCATTTTAC  | TCTATAAACA  | TTTTACCACA  | AATAATAGGA  |
| 3901 | TCCTCTAGAT  | ATTTAATATT  | ATATCTAACA  | ACAACAAAAA  | AATTTAACGA  | TGTATGGCCA  |
| 3961 | GAAGTATTTT  | CTACTAATAA  | AGATAAAGAT  | AGTCTATCTT  | ATCTACAAGA  | TATGAAAGAA  |
| 4021 | GATAATCATT  | TAGTAGTAGC  | TACTAATATG  | GAAAGAAATG  | TATACAAAAA  | CGTGGAAAGCT |
| 4081 | TTTATATTAA  | ATAGCATATT  | ACTAGAAGAT  | TTAAAATCTA  | GACTTAGTAT  | AACAAAACAG  |
| 4141 | TTAAATGCCA  | ATATCGATTTC | TATATTTTCA  | CATAACAGTA  | GTACATTAAT  | CAGTGATATA  |
| 4201 | CTGAAACGAT  | CTACAGACTC  | AACATATGCA  | GGAATAAGCA  | ATATGCCAAT  | TATGTCTAAT  |
| 4261 | ATTTTAACTT  | TAGAACTAAA  | ACGTTCTACC  | AATACTAAAA  | ATAGGATACG  | TGATAGGCTG  |
| 4321 | TTAAAAGCTG  | CAATAAATAG  | TAAGGATGTA  | GAAGAAATAC  | TTTGTTCTAT  | ACCTTCGGAG  |
| 4381 | GAAAGAACCT  | TAGAACAACCT | TAAGTTTAAT  | CAAACCTTGTA | TTTATGAAGG  | TACC        |

## Fig. 4

Figure 4 DNA sequence of the coding region of FHV gB with modified T5NT motifs.

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1  ATGTCCACTC GTGGCGATCT TGGGAAGCGG CGACGAGGGA GTCGTTGGCA GGGACACAGT
61 GGCTATTTTC GACAGAGATG TTTTTCCT TCTCTACTCG GTATTGCAGC GACTGGCTCC
121 AGACATGGTA ACGGATCGTC GGGATTAACC AGACTAGCTA GATATGTTTC ATTTATCTGG
181 ATCGTACTAT TCTTAGTCGG TCCCCGTCCA GTAGAGGGTC AATCTGGAAG CACATCGGAA
241 CAACCCCGGC GGACTGTAGC TACCCCTGAG GTAGGGGGTA CACCACCAA ACCAACTACA
301 GATCCCACCG ATATGTCGGA TATGAGGGAA GCTCTCCGTG CGTCCCAAAT AGAGGCTAAC
361 GGACCATCGA CTTTCTATAT GTGTCCACCA CCTTCAGGAT CTACTGTCTG GCGTTTAGAG
421 CCACCACGGG CCTGTCCAGA TTATAAACTA GGGAAAAATF TTACCGAGGG TATAGCTGTA
481 ATATTTAAAG AAAATATAGC GCCATATAAA TTCAAGGCAA ATATATACTA TAAAAACATT
541 ATTATGACAA CGGTATGGTC TGGGAGTTCC TATGCCGTTA CAACCAACCG ATATACAGAC
601 AGGGTTCCCG TGAAAGTTCA AGAGATTACA GATCTCATAG ATAGACGGGG TATGTGCCTC
661 TCGAAAGCTG ATTACGTTCT TAACAATTAT CAATTTACGG CCTTTGATCG AGACGAGGAT
721 CCCAGAGAAC TGCCTCTGAA ACCCTCCAAG TTCAACACTC CAGAGTCCCG TGGATGGCAC
781 ACCACCAATG AAACATACAC AAAGATCGGT GCTGCTGGAT TTCACCACTC TGGGACCTCT
841 GTAAATTGCA TCGTAGAGGA AGTGGATGCA AGATCTGTAT ATCCATATGA CTCATTTGCT
901 ATCTCCACTG GTGACGTGAT TCACATGTCT CCATTCTTTG GGCTGAGGGA TGGAGCCCAT
961 GTAGAACATA CTAGTTATTC TTCAGACAGA TTTCAACAAA TCGAGGGGATA CTATCCAATA
1021 GACTTGGATA CGCGATTACA ACTGGGGGCA CCAGTTTCTC GCAATTTTTT GGAACCTCCG
1081 CATGTGACAG TGGCCTGGAA CTGGACCCCA AAGTCTGGTC GGGTATGTAC CTTAGCCAAA
1141 TGGAGGGAAA TAGATGAAAT GCTACGCGAT GAATATCAGG GCTCCTATAG ATTTACAGCC
1201 AAGACCATAT CCGCTACTTT CATCTCCAAT ACTTCACAAT TTGAAATCAA TCGTATCCGT
1261 TTGGGGGACT GTGCCACCAA GGAGGCAGCC GAAGCCATAG ACCGGATTTA TAAGAGTAAA
1321 TATAGTAAAA CTCATATTCA GACTGGAACC CTGGAGACCT ACCTAGCCCG TGGGGGATTT
1381 CTAATAGCTT TCCGTCCCAT GATCAGCAAC GAAC TAGCAA AGTTATATAT CAATGAATTA
1441 GCACGTTCCT ATCGCACGGT AGATCTCAGT GCATCCTCA ATCCATCTGG GGAACAGTA
1501 CAACGAACCTA GAAGATCGGT CCCATCTAAT CAACATCATA GGTGCGGGCG CAGCACAATA
1561 GAGGGGGGTA TAGAAACCGT GAACAATGCA TCACTCCTCA AGACCACCTC ATCTGTGGAA
1621 TTCGCAATGC TACAATTTGC CTATGACTAC ATACAAGCCC ATGTAAATGA AATGTTGAGT
1681 CGGATAGCCA CTGCCTGGTG TACACTTCAG AACCGCGAAC ATGTGCTGTG GACAGAGACC
1741 CTAAAACCTA ATCCCGGTGG GGTGGTCTCG ATGGCCCTAG AACGTCGTGT ATCCGCGCGC
1801 CTAATTGGAG ATGCCGTCGC CGTAACACAA TGTGTTAACA TTTCTAGCGG ACATGTCTAT
1861 ATCCAAAATT CTATGCGGGT GACGGGTTC TCAACGACAT GTTACAGCCG CCCTCTTGTT
1921 TCCTTCCGTG CCCTCAATGA CTCCGAATAC ATAGAAGGAC AACTAGGGGA AAACAATGAA
1981 CTTCTCGTGG AACGAAAAC AATTGAGCCT TGCCTGTCA ATAATAAGCG GTATTTTAAG
2041 TTTGGGGCAG ATTATGTATA TTTTGAGGAT TATGCGTATG TCCGTAAAGT CCCGCTATCG
2101 GAGATAGAAC TGATAAGTGC GTATGTGAAT TTAAATCTTA CTCTCCTAGA GGATCGTGAA
2161 TTTCTCCAC TCGAAGTTTA TACACGAGCT GAGCTGGAAG ATACCGGCCT TTTGGACTAC
2221 AGCGAGATTC AACGCCGCAA CCAACTCCAC GCCTTAAAT TTTATGATAT AGACAGCATA
2281 GTCAGAGTGG ATAATAATCT TGTCATCATG CGTGGTATGG CAAATTTCTT TCAGGGACTC
2341 GGGGATGTGG GGGCTGGTTT CGGCAAGGTG GTCTTAGGGG CTGCGAGTGC GGTAATCTCA
2401 ACAGTATCAG GCGTATCATC ATTTCTAAAC AACCCATTTG GAGCATTTGG CGTGGGACTG
2461 TTAATATTAG CTGGCATCGT CGCAGCATTC CTGGCATATC GCTATATATC TAGATTACGT
2521 GCAAATCCAA TGAAAGCCTT ATATCTGTG ACGACTAGGA ATTTGAAACA GAGCCTAAGA
2581 GCCCCTCAA CGGCTGGTGG GGATAGCGAC CCGGAGTTCG ATGACTTCGA TAGGAAAAG
2641 CTAATGCAGG CAAGGGAGAT GATAAAATAT ATGTCCCTCG TATCGGCTAT GGAGCAACAA
2701 GAACATAAGG CGATGAAAAA GAATAAGGGC CCAGCGATCC TAACGAGTCA TCTCACTAAC
2761 ATGGCCCTCC GTCGCCGTGG ACCTAAATAC CAACGCCTCA ATAATCTTGA TAGCGGTGAT

```

*Fig. 4 (co + d)*

2821 GATACTGAAA CAAATCTTGT CTAA



*Fig. 5*

Figure 5 DNA sequence of the the H6 promoted FHV gB donor plasmid  
pC3H6FHVb.

H6 promoter: 3958 - 3835

FHV gB coding region: 3834 - 991

C3 left arm: 15 - 939

C3 right arm: 4056 - 6628

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1  GCGGCCGCGT  CGACATGCAT  TGTTAGTTCT  GTAGATCAGT  AACGTATAGC  ATACGAGTAT
61 AATTATCGTA  GGTAGTAGGT  ATCCTAAAAT  AAATCTGATA  CAGATAATAA  CTTTGTAAT
121 CAATTCAGCA  ATTTCTCTAT  TATCATGATA  ATGATTAATA  CACAGCGTGT  CGTTATTTTT
181 TGTACGATA  GTATTTCTAA  AGTAAAGAGC  AGGAATCCCT  AGTATAATAG  AAATAATCCA
241 TATGAAAAAT  ATAGTAATGT  ACATATTTCT  AATGTTAACA  TATTTATAGG  TAAATCCAGG
301 AAGGGTAATT  TTTACATATC  TATATACGCT  TATTACAGTT  ATTAATAATA  TACTTGCAAA
361 CATGTTAGAA  GTAAAAAAGA  AAGAACTAAT  TTTACAAAGT  GCTTTACCAA  AATGCCAATG
421 GAAATTACTT  AGTATGTATA  TAATGTATAA  AGGTATGAAT  ATCACAACA  GCAAATCGGC
481 TATCCCAAG  TTGAGAAACG  GTATAATAGA  TATATTTCTA  GATACCATTA  ATAACCTTAT
541 AAGCTTGACG  TTTCCTATAA  TGCCTACTAA  GAAAACCTAGA  AGATACATAC  ATACTAACGC
601 CATACGAGAG  TAACTACTCA  TCGTATAACT  ACTGTTGCTA  ACAGTGACAC  TGATGTTATA
661 ACTCATCTTT  GATGTGGTAT  AAATGTATAA  TAACTATATT  ACACTGGTAT  TTTATTTTCAG
721 TTATATACTA  TATAGTATTA  AAAATTATAT  TTGTATAATT  ATATTATTAT  ATTCAGTGTA
781 GAAAGTAAAA  TACTATAAAT  ATGTATCTCT  TATTTATAAC  TTATTAGTAA  AGTATGTACT
841 ATTCAGTTAT  ATTGTTTTAT  AAAAGCTAAA  TGCTACTAGA  TTGATATAAA  TGAATATGTA
901 ATAAATTAGT  AATGTAGTAT  ACTAATATTA  ACTCACATTT  GACTAATAAG  CTATAAAAC
961 CCGGGCTGCA  GCGCGGAAG  CTTACAAAAA  TTAGACAAGA  TTTGTTTCAG  TATCATCACC
1021 GCTATCAAGA  TTATTGAGGC  GTTGGTATTT  AGGTCCACGG  CGACGGAGGG  CCATGTTAGT
1081 GAGATGACTC  GTTAGGATCG  CTGGGCCCTT  ATTCTTTTTT  ATCGCCTTAT  GTTCTTGTTG
1141 CTCCATAGCC  GATACGAGGG  ACATATATTT  TATCATCTCC  CTGTCCTGCA  TTAGCTTTTC
1201 CTCATCGAAG  TCATCGACTC  CCGGGTCGCT  ATCCCCACCA  GCCGTTGAGC  GGGCTCTTAG
1261 CGTCTGTTTC  AAATTCCTAG  TCGTCACAGG  ATATAAGGCT  TTCATTGGAT  TTGCACGTAA
1321 TCTAGATATA  TAGCGATATG  CCAGGAATGC  TGCGACGATG  CCAGCTAATA  TTAACAGTCC
1381 CACGGCCAAT  GCTCCAAATG  GGTGTTTAG  AAATGATGAT  ACGCCTGATA  CTGTTGAGAT
1441 TACCGCACTC  GCAGCCCCTA  AGACCACCTT  GCCGAAACCA  GCCCCACAT  CCCCAGGTCC
1501 CTGAAAGAAA  TTTGCCATAC  CACGCATGAT  GACAAGATTA  TTATCCACTC  TGACTATGCT
1561 GTCTATATCA  TAAAATTTTA  AGGCGTGGAG  TTGGTTGCGG  CGTTGAATCT  CGCTGTAGTC
1621 CAAAAGGCCG  GTATCTTCCA  GCTCAGCTCG  TGTATAAACT  TCGAGTGGGA  GAAATTCACG
1681 ATCCTCTAGG  AGAGTAAGAT  TTAAATTCAC  ATACGCACTT  ATCAGTTCTA  TCTCCGATAG
1741 CGGGACTTTA  CGGACATACG  CATAATCCTC  AAAATATACA  TAATCTGCCC  CAAACTTAAA
1801 ATACCGCTTA  TTATTGACAG  TGCAAGGCTC  AATTAGTTTT  CGTTCCACGA  GAAGTTCATT
1861 GTTTTCCCTT  AGTTGTCCTT  CTATGTATTC  GGAGTCATTG  AGGGCACGGA  AGGAAACAAG
1921 AGGGCGGCTG  TAACATGTCT  TTGATGAACC  CGTCACCCGC  ATAGAATTTT  GGATATAGAC
1981 ATGTCCGCTA  GAAATGTTAA  CACATTGTGT  TACGGCGACG  GCATCTCCAA  GTAGGCGCGC
2041 GGATACACGA  CGTTCTAGGG  CCATCGAGAC  CACCCACCG  GGATTGAGTT  TTAGGGTCTC
2101 TGTCCACAGC  ACATGTTCTG  GGTCTGAAG  TGTACACCAG  GCAGTGGCTA  TCCGACTCAA
2161 CATTTCATTT  ACATGGGCTT  GTATGTAGTC  ATAGGCAAAT  TGTAGCATTG  CGAATTCAC
2221 AGATGAGGTG  GTCTTGAGGA  GTGATGCATT  GTTCACGGTT  TCTATACCCC  CCTCTATTGT
```

## Fig. 5 (cont.)

2281 GCTGCGCCGC GACCTATGAT GTTGATTAGA TGGGACCGAT CTTCTAGTTC GTTGACTGT  
2341 TTCCCCAGAT GGATTGAGGA GTGCACTGAG ATCTACCGTG CGATTGGAAC GTGCTAATTC  
2401 ATTGATATAT AACTTTGCTA GTTCGTTGCT GATCATGGGA CGGAAAGCTA TTAGAAATCC  
2461 CCCACGGGCT AGGTAGGTCT CCAGGGTTCC AGTCTGAATA TGAGTTTAC TATATTTACT  
2521 CTTATAAATC CGGTCTATGG CTTGCGCTGC CTCCTTGGTG GCACAGTCCC CCAAACGGAT  
2581 ACGATTGATT TCAAATTGTG AAGTATTGGA GATGAAAGTA GCGGATATGG TCTTGGCTGT  
2641 AAATCTATAG GAGCCCTGAT ATTCATCGCG TAGCATTTC TCTATTTCCC TCCATTTGGC  
2701 TAAGGTACAT ACCCGACCAG ACTTTGGGGT CCAGTTCCAG GCCACTGTCA CATGCGGAGT  
2761 TTCCAAAAA TTGCGAGAAA CTGGTGCCCC CAGTTGTAAT CGCGTATCCA AGTCTATTGG  
2821 ATAGTATCCC TCGATTTGTT GAAATCTGTC TGAAGAATAA CTAGTATGTT CTACATGGGC  
2881 TCCATCCCTC AGCCCAAAGA ATGGAGACAT GTGAATCACG TCACCAGTGG AGATAGCAAA  
2941 TGAGTCATAT GGATATACAG ATCTTGCAAT CACTTCTCT ACGATGCAAT TTACAGAGGT  
3001 CCCAGAGTGG TGAATCCAG CAGCACCGAT CTTTGTGTAT GTTTCATTGG TGGTGTGCCA  
3061 TCCACGGGAC TCTGGAGTGT TGAACCTGGA GGGTTTCAGA GGCAGTTCTC TGGGATCCTC  
3121 GTCTCGATCA AAGGCCGTAA ATTGATAATT GTTACGAACG TAATCAGCTT TCGAGAGGCA  
3181 CATACCCCGT CTATCTATGA GATCTGTAAT CTCTTGAAC TTACCGGGAA CCCTGTCTGT  
3241 ATATCGGTTG GTTGTAAACG CATAGGAACT CCCAGACCAT ACCGTTGTCA TAATAATGTT  
3301 TTTATAGTAT ATATTTGCCT TGAATTTATA TGGCGCTATA TTTTCTTTAA ATATTACAGC  
3361 TATACCCCTC GTAAAATTTT TCCCTAGTTT ATAATCTGGA CAGGCCCCGTG GTGGCTCTAA  
3421 ACGCACGACA GTAGATCCTG AAGGTGGTGG ACACATATAG AAAGTCGATG GTCCGTTAGC  
3481 CTCTATTTGG GACGCACGGA GAGCTTCCCT CATATCCGAC ATATCGGTGG GATCTGTAGT  
3541 TGGTTTTGGT GGTGTACCCC CTACCTCAGG GGTAGCTACA GTCCGCCGGG GTTGTTCGGA  
3601 TGTGCTTCCA GATTGACCC CTACTGGACG GGGACCGACT AAGAATAGTA CGATCCAGAT  
3661 AAATGAAACA TATCTAGCTA GTCTGGTTAA TCCCGACGAT CCGTTACCAT GTCTGGAGCC  
3721 AGTCGCTGCA ATACCGAGTA GAGAAGGGA AAAACATCTC TGTCGAAAAT AGCCACTGTG  
3781 TCCCTGCCAA CGACTCCCTC GTCGCCGCTT CCAAGATCG CCACGAGTGG GTTCAATGAT  
3841 ACAAACCTAA CGGATATCGC GATAATGAAA TAATTTATGA TTATTTCTCG CTTTCAATTT  
3901 AACACAACCC TCAAGAACCT TTGTATTTAT TTTCACTTTT TAAGTATAGA ATAAAGAAGC  
3961 TCTAATTAAT TAAGCTACAA ATAGTTTCGT TTTACCTTG TCTAATAACT AATTAATTAA  
4021 CCCGGATCGA TCCCGATTTT TATGACTAGT TAATCAAATA AAAAGCATAC AAGCTATTGC  
4081 TTCGCTATCG TTACAAAATG GCAGGAATTT TGTGTAACT AAGCCACATA CTTGCCAATG  
4141 AAAAAAATAG TAGAAAGGAT ACTATTTTAA TGGGATTAGA TGTTAAGGTT CTTGGGATT  
4201 ATAGTAACTG GGCATCTGTT AACTTTTACG ACGTTAGGTT AGATACTGAT GTTACAGATT  
4261 ATAATAATGT TACAATAAAA TACATGACAG GATGTGATAT TTTTCCTCAT ATAACCTTG  
4321 GAATAGCAAA TATGGATCAA TGTGATAGAT TTGAAAATTT CAAAAAGCAA ATAACGTATC  
4381 AAGATTTACA GACTATTTCT ATAGTCTGTA AAGAAGAGAT GTGTTTTCTT CAGAGTAACG  
4441 CCTCTAAACA GTTGGGAGCG AAAGGATGCG CTGTAGTTAT GAAACTGGAG GTATCTGATG  
4501 AACTTAGAGC CCTAAGAAAT GTTCTGCTGA ATGCGGTACC CTGTTCTGAG GACGTGTTTG  
4561 GTGATATCAC AGTAGATAAT CCGTGGAATC CTCACATAAC AGTAGGATAT GTTAAGGAGG  
4621 ACGATGTCGA AAACAAGAAA CGCCTAATGG AGTGCATGTC CAAGTTTAGG GGGCAAGAAA  
4681 TACAAGTTCT AGGATGGTAT TAATAAGTAT CTAAGTATTT GGTATAATTT ATTAATAGT  
4741 ATAATTATAA CAAATAATAA ATAACATGAT AACGGTTTTT ATTAGAATAA AATAGAGATA  
4801 ATATCATAAT GATATATAAT ACTTCATTAC CAGAAATGAG TAATGGAAGA CTTATAAATG  
4861 AACTGCATAA AGCTATAAGG TATAGAGATA TAAATTTAGT AAGGTATATA CTTAAAAAAT  
4921 GCAAAATACAA TAACGTAAAT ATACTATCAA CGTCTTTGTA TTTAGCCGTA AGTATTTCTG  
4981 ATATAGAAAT GGTAAATTA TTACTAGAAC ACGGTGCCGA TATTTTAAAA TGTAATAATC  
5041 CTCCTCTTCA TAAAGCTGCT AGTTTAGATA ATACAGAAAT TGCTAAACTA CTAATAGATT  
5101 CTGGCGCTGA CATAGAACAG ATACATTCTG GAAATAGTCC GTTATATATT TCTGTATATA  
5161 GAAACAATAA GTCATTAACT AGATATTTAT TAAAAAAGG TGTTAATTTG AATAGATTCT  
5221 TTCTAAATTA TTACGATGTA CTGTATGATA AGATATCTGA TGATATGTAT AAAATATTTA  
5281 TAGATTTTAA TATTGATCTT AATATACAAA CTAGAAATTT TGAAACTCCG TACATTTACG  
5341 CTATAAAGTA TAAGAATATA GATTTAATTA GGATATTGTT AGATAATAGT ATTAATAATG  
5401 ATAAAAGTTT ATTTTTGCAT AAACAGTATC TCATAAAGGC ACTTAAAAAT AATTGTAGTT  
5461 ACGATATAAT AGCGTTACTT ATAAATCACG GAGTGCCTAT AAACGAACAA GATGATTTAG

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Fig. 5 (cor. 41)

5521 GTAAAACCCC ATTACATCAT TCGGTAATTA ATAGAAGAAA AGATGTAACA GCACTTCTGT  
5581 TAAATCTAGG AGCTGATATA AACGTAATAG ATGACTGTAT GGGCAGTCCC TTACATTACG  
5641 CTGTTTCACG TAACGATATC GAAACAACAA AGACACTTTT AGAAAGAGGA TCTAATGTTA  
5701 ATGTGGTTAA TAATCATATA GATACCGTTC TAAATATAGC TGTTGCATCT AAAAAACAAA  
5761 CTATAGTAAA CTTATTACTG AAGTACGGTA CTGATACAAA GTTGGTAGGA TTAGATAAAC  
5821 ATGTTATTCA CATAGCTATA GAAATGAAAG ATATTAATAT ACTGAATGCG ATCTTATTAT  
5881 ATGGTTGCTA TGTAACGTC TATAATCATA AAGGTTTCAC TCCTCTATAC ATGGCAGTTA  
5941 GTTCTATGAA AACAGAATTT GTTAAACTCT TACTTGACCA CGGTGCTTAC GTAAATGCTA  
6001 AAGCTAAGTT ATCTGGAAAT ACTCCTTTAC ATAAAGCTAT GTTATCTAAT AGTTTTAATA  
6061 ATATAAAATT ACTTTTATCT TATAACGCCG ACTATAATTC TCTAAATAAT CACGGTAATA  
6121 CGCCTCTAAC TTGTGTTAGC TTTTITAGATG ACAAGATAGC TATTATGATA ATATCTAAAA  
6181 TGATGTTAGA AATATCTAAA AATCCTGAAA TAGCTAATTC AGAAGGTTTT ATAGTAAACA  
6241 TGGAACATAT AAACAGTAAT AAAAGACTAC TATCTATAAA AGAATCATGC GAAAAAGAAC  
6301 TAGATGTTAT AACACATATA AAGTTAAATT CTATATATTC TTTTAATATC TTTCTTGACA  
6361 ATAACATAGA TCTTATGGTA AAGTTCGTAA CTAATCCTAG AGTTAATAAG ATACCTGCAT  
6421 GTATACGTAT ATATAGGGAA TTAATACGGA AAAATAAATC ATTAGCTTTT CATAGACATC  
6481 AGCTAATAGT TAAAGCTGTA AAAGAGAGTA AGAATCTAGG AATAATAGGT AGGTTACCTA  
6541 TAGATATCAA ACATATAATA ATGGAACATAT TAAGTAATAA TGATTTACAT TCTGTTATCA  
6601 CCAGCTGTTG TAACCCAGTA GTATAAAG

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Fig. 6

## POL/NEF epitopes

10 20 30 40 50 60 70 80 90 100 110  
TTTTTTCAT TATTTAGAAA TTATGCATTT TAGATCTTTA TAAGCGGCGG TGATTAACCTA GTCATAAAAA CCCGGGATCG ATTCTAGACT CGAGGGTACC GGATCTTAAT  
AAAAAAGTA ATAAATCTTT AATACGTAAA ATCTAGAAAT ATTCGCGCGC ACTAATTGAT CAGTATTTTT GGGCCCTAGC TAAGATCTGA GCTCCCATGG CCTAGAATTA

120 130 140 150 160 170 180 190 200 210 220  
TAATTAGTCA TCAGGCAGGG CGAGAACGAG ACTATCTGCT CGTTAATTAA TTAGGTCGAC GGATCCCCCA ACAAACCTA ATCAGCTATC GGGGTAAATT AATTAGTTAT  
ATTAATCAGT AGTCCGTCCT GCTCTTGCTC TGATAGACGA GCAATTAATT AATCCAGCTG CCTAGGGGGT TGTITTTGAT TAGTCGATAG CCCCATTAA TTAATCAATA

230 240 250 260 270 280 290 300 310 320 330  
TAGACAAGGT GAAACGAAA CTATTTGTAG CTTAATTAAT TAGAGCTTCT TTATCTATA CTAAAAAGT GAAATAAAT ACAAAGGTTT TTGAGGGTTG TGTTAAATTG  
ATCTGTTCCA CTTTGTCTTT GATAAACATC GAATTAATTA ATCTCGAAGA AATAAGATAT GAATTTTCA CTTTATTTA TGTTCCTAAG AACTCCCAAC ACAATTTAAC  
H6 promoter

340 350 360 370 380 390 400 410 420 430  
AAAGCGAGAA ATAATCATAA ATTATTTTCAT TATCGCGATA TCCGTTAAGT TTGTATCGTA ATG CCA CTA ACA GAA GCA GAG CTA GAA CTG GCA GAA AAC  
TTTCGCTCTT TATTAGTATT TAATAAAGTA ATAGCGCTAT AGGCAATTCA AACATAGCAT TAC GGT GAT TGT CTT CTT CGT CTC GAT CTT GAC CGT CTT TTG  
H6 promoter Met Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn  
POL/NEF Epitopes

440 450 460 470 480 490 500 510 520  
AGA GAG ATT CTA AAA GAA CCA GTA CAT GGA GTG TAT TAT GAC CCA TCA AAA GAC TTA ATA GCA GAA ATA CAG AAG CAG GGG CAA GGC CAA  
TCT CTC TAA GAT TTT CTT GGT CAT GTA CCT CAC ATA ATA CTG GGT AGT TTT CTG AAT TAT CGT CTT TAT GTC TTC GTC CCC GTT CCG GTT  
Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln  
POL/NEF Epitopes

530 540 550 560 570 580 590 600 610  
TGG ACA TAT CAA ATT TAT CAA GAG CCA TTT AAA AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT AGA TTA GCA TTT CAT CAC GTA  
ACC TGT ATA GTT TAA ATA GTT CTC GGT AAA TTT TTA GAC TTT TGT CCT TAC CTC ACC TCT AAA CTA AGA TCT AAT CGT AAA GTA GTG CAT  
Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Met Glu Trp Arg Phe Asp Ser Arg Leu Ala Phe His His Val  
POL/NEF Epitopes

620 630 640 650 660 670 680 690 700  
GCT AGA GAA TTA CAT CCT GAA TAT TTT AAA AAT TGT AAG CTT ATG GCA ATA TTC CAA AGT AGC ATG ACA AAA ATC TTA GAG CCT TTT AGA  
CGA TCT CTT AAT GTA GGA CTT ATA AAA TTT TTA ACA TTC GAA TAC CGT TAT AAG GTT TCA TCG TAC TGT TTT TAG AAT CTC GGA AAA TCT  
Ala Arg Glu Leu His Pro Glu Tyr Phe Lys Asn Cys Lys Leu Met Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg  
POL/NEF Epitopes

710 720 730 740 750 760 770 780 790  
AAA CAA AAT CCA GAC CAT GTT ATC TAT CAA TAC ATG GAT GAT TTG TAT GTA GGA TCT GAC TTA GAA ATA GGG CAG CAT AGA ACA AAA ATA  
TTT GTT TTA GGT CTG TAT CAA TAG ATA GTT ATG TAC CTA CTA AAC ATA CAT CCT AGA CTG AAT CTT TAT CCC GTC GTA TCT TGT TTT TAT  
Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile  
POL/NEF Epitopes

800 810 820 830 840 850 860 870 880  
GAG GAG CTG AGA CAA CAT CTG TTG AGG TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA ACA CCT CAA GTA CCT TTA AGA CCA ATG ACT  
CTC CTC GAC TCT GTT GTA GAC AAC TCC ACC CCT GAA TGT TGG TAC CAT CCA AAA GGT CAT TGT GGA GTT CAT GGA AAT TCT GGT TAC TGA  
Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Met Val Gly Phe Pro Val Thr Pro Gln Val Pro Leu Arg Pro Met Thr  
POL/NEF Epitopes

## Fig. 6 (cont'd)

890 900 910 920 930 940 950 960 970  
\* \* \* \* \*  
TAC AAA GCA GCT GTA GAT CTT TCT CAC TTT TTA AAA GAA AAA GGA GGT TTA GAA GGG CTA ATT CAT TCT CAA CGA AGA CAA GAT ATT CTT  
ATG TTT CGT CGA CAT CTA GAA AGA GTG AAA AAT TTT CTT TTT CCT CCA AAT CTT CCC GAT TAA GTA AGA GTT GCT TCT GTT CTA TAA GAA  
Tyr Lys Ala Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu  
POL/NEF Epitopes >

980 990 1000 1010 1020 1030 1040 1050 1060  
\* \* \* \* \*  
GAT TTG TGG ATT TAT CAT ACA CAA GGA TAT TTT CCT GAT TGG CAG AAT TAC ACA CCA GGA CCA GGA GTC AGA TAC CCA TTA ACC TTT GGT  
CTA AAC ACC TAA ATA GTA TGT GTT CCT ATA AAA GGA CTA ACC GTC TTA ATG TGT GGT CCT GGT CCT CAG TCT ATG GGT AAT TGG AAA CCA  
Asp Leu Trp Ile Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly  
POL/NEF Epitopes >

1070 1080 1090 1100 1110 1120 1130 1140 1150  
\* \* \* \* \*  
TGG TGC TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA AAA TTA AAG CCA GGA ATG GAT GGC CCA AAA GTT AAA CAA TGG CCA TTG  
ACC ACG ATG TTC GAT CAT GGT TAC TAA CTC TGA CAT GGT CAT TTT AAT TTC GGT CCT TAC CTA CCG GGT TTT CAA TTT GTT ACC GGT AAC  
Trp Cys Tyr Lys Leu Val Pro Met Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu  
POL/NEF Epitopes >

1160 1170 1180 1190 1200 1210 1220 1230 1240  
\* \* \* \* \*  
ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAG ATG GAA AAG GAA GGG AAA ATT TCA AAA ATT GGG CCT TAA TTTTCT  
TGT CTT CTT TTT TAT TTT CGT AAT CAT CTT TAA ACA TGT CTC TAC CTT TTC CTT CCC TTT TAA AGT TTT TAA CCC GGA ATT AAAAAGA  
Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro  
POL/NEF Epitopes >

1250 1260 1270 1280 1290 1300 1310 1320 1330 1340 1350  
\* \* \* \* \*  
GCAGCCCCGGG GGATCCTTTT TATAGCTAAT TAGTCACGTA CCTTTGAGAG TACCACTTCA GCTACCTCTT TTGTGTCTCA GAGTAACCTT CTTTAATCAA TTCAAAACA  
CGTCGGGCCC CCTAGGAAAA ATATCGATTA ATCAGTGCAT GGAACTCTC ATGGTGAAGT CGATGGAGAA AACACAGAGT CTCATTGAAA GAAATTAGTT AAGGTTTTGT

Fig. 7

gag (+ pro) and gp120 (+ transmembrane)

FEATURES From To/Span Description

| FEATURES | From | To/Span  | Description                          |
|----------|------|----------|--------------------------------------|
| frag     | 1    | 56       | C3 flanking arm                      |
| frag     | 162  | 76 (C)   | HIV1 (111B) env transmembrane region |
| frag     | 1728 | 163 (C)  | HIV1 (MN) gp120 gene                 |
| frag     | 1853 | 1729 (C) | vaccinia H6 promoter                 |
| frag     | 1925 | 1983     | vaccinia I3L promoter                |
| frag     | 1984 | 3746     | HIV1 (111B) gag/pro gene             |
| frag     | 3753 | 3808     | C3 flanking arm                      |

10 20 30 40 50 60 70 80 90 100 110 120  
 TAATGTAGTATACTAATATTAACACATTTGACTAATAGCTATAAAAAACCCGGGATCGATTCTAGAATAAAAAATTATCCCTGCCTAACTCTATTCTACACAGAGAGTACAGCAAAAAAC  
 ATTACATCATATGATTATAATTGAGTGTAACCTGATTATCGATATTTTGGGCCCTAGCTAAGATCTTATTTTAAATAGGACGGATTGAGATAAGTGATGCTCTCATGTCGTTTTTG  
 C3 FLANKING ARM > G Q R V R N V V S L V A F V  
 < HIV1 (111B) ENV TRANSMEMBRANE REGION

130 140 150 160 170 180 190 200 210 220 230 240  
 TATTCTAAACCTACCAAGCCTCCTACTATCATTATGAATAATCTTTTTCTCTGCACCACTCTCTCTTTGCCTTGGTGGTGCTACTCCTAATGGTCAATTGTTACTACTTTATA  
 ATAAGAATTTGGATGGTTCGGAGGATGATAGTAATCTTATTAGAAAAAGAGAGACGCTGGTGAGAGAGAAACCGAACCCACCGATGAGGATTACCAAGTTAACAATGATGAAATAT  
 I R L G V L G G V I M I F L R K E R Q V V R R K A K T P A V G L P E I T V V K Y  
 < HIV1 (111B) ENV TRANSMEMBRANE REGION  
 HIV1 (MN) GP120 GENE

250 260 270 280 290 300 310 320 330 340 350 360  
 TTTATATAATTCACCTTCTCCAATTGTCCTCATATCTCTCCTCCAGGTCTGAAGATCTCGGTGTCGTTGCTGTCCTTACCACCATCTCTTGTAAATAGTAGCCCTGTAATATT  
 AAATATATTAAGTGAAGAGGTTAACAGGGAGTATAGAGGAGGAGGTCCAGACTTCTAGAGCCACAGCAAGCACAGGCACAGGAATGGTGGTAGAGAAATATCATCGGGACATTATAA  
 K Y L E S R W N D R M D G G G P R F I E T D N T D T D K G G D R T L L L G T I N  
 < HIV1 (MN) GP120 GENE

370 380 390 400 410 420 430 440 450 460 470 480  
 TGATGAACATCTAATTTGTCCTTCAATGGGAGGGGCATATATTGCTTTTCTCTACTTCTGCCACATGTTTATAATTTGTTTATTTTGCATTGAAGTGTGATTTGTTATTTGACCCGTG  
 ACTACTTGTAGATTAAACAGGAAGTTACCTTCCCGTATATAACGAAAGGATGAAGGACGGTGTACAAATATTAACAAAAATAAACGTAACCTCACACATAACAATAAACTGGGACA  
 S S C R I Q G E I P P A Y I A K G V E Q W M N I I Q K I K C Q L T I N N N S G T  
 < HIV1 (MN) GP120 GENE

490 500 510 520 530 540 550 560 570 580 590 600  
 AGTATTATTTCCAAGTATTATTACCAATTCAGTACTATTAACAGTGGTGATGAATTACAGTAGAAGAAATCCCTCCACAATTAACCTGTGCATTACAATTTCTGGGTCCCTCCTGA  
 TCATAATAAGGTTTATAATAATGGTAAGGTTTCATGATAATTTGTCCACTACTTAATGTCTCTTTAAAGGGGAGGTGTTAATTTTGACACGTAATGTTAAAGACCCAGGGGAGGACT  
 T N N W T N N G N W T S N F L P S S N C Y F F E G G C N F S H M V I E P D G G S  
 < HIV1 (MN) GP120 GENE

610 620 630 640 650 660 670 680 690 700 710 720  
 GGATTGATTAAGACTATTGTTTTATTCTTAAATTTGCTTTTAAATTTGCTAACTATCTGTCTTAAAGTGTCAATTTTGTCTACTAATGTTACAATGTGCTTGTCTTATAGTTCC  
 CTTAACTAATTTCTGATAACAAAAAAGAATTTAACAAGAAAAATTAACGATTGATAGACAGAAATTCACAGTAAGGTAACGAGATGATTACAATGTTACACGACAGAAATATCAAGG  
 S Q N F V I T K N K F Q E K L K S V I Q R L T D N W K A R S I N C H A Q R I T G  
 < HIV1 (MN) GP120 GENE

730 740 750 760 770 780 790 800 810 820 830 840  
 TATTATATTTTGTGTTATAAAATGCTCTCCCTGGTCTATATGTATCCCTTTTCTTTTATTGTAGTTGGGCTTGTACAATTAATTTGTACAGATTCATTGATGACTATGATGGT  
 ATAATATAAAAAACAATATTTTACGAGAGGGACCGGATATACATAGGAAAAAGAAAAATACATCAACCCAGAACATGTTAATTAACATGTCTAAGTAAGTCTACATGATACTACCA  
 I I N K T T Y F A R G P G I H I R K R K N Y N P R T C N I Q V S E N L H V I I T  
 < HIV1 (MN) GP120 GENE

850 860 870 880 890 900 910 920 930 940 950 960  
 TTTAGCATTTATCATTGAAATCTCAGATCTAATTAATCTCTCTCTGCTAGACTGCCATTTAACAGCAGTGTGAGTTGATACTACTGGCCTAATTCATGTGTACATTGTACTGTGCT  
 AAATCGTAATAGTAACCTTAAGAGTCTAGATTAATGATGGAGAAGAAGACGATCTGACGGTAAATTTGCTGCAACTCAACTATGATGACCGGATTAAGGTACACATGTAAACATGACACGA  
 K A N D N F N E S R I V V E E E A L S G N L L Q T S V V P R I G H T C Q V T S  
 < HIV1 (MN) GP120 GENE

Fig. 7 (cont'd)

970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080  
GACATTTTACATGATCCTTTCCACTGAACCTTTTATCGTTACACTTTAGAATCGCAAAACAGCGGGGACCAATAGTGTATGGGAATGGCTCAAAGGATATCTTTGGACAAGCTTG  
ETGTAAAAATGTACTAGGAAAAGGTGACTTGAAAAATAGCAATGTGAAATCTTAGCGTTTGGTCGGCCCCGTGTATCACATACCTTAACCGAGTTTCTATAGAAAACCTGTTGCAAC  
V N K C S G K G S F K K D N C K L I A F G A P A C Y H I P I P E F S I K P C A Q  
< HIV1 (MN) GP120 GENE

1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200  
TGTAATGACTGAGGTATTACAACCTATCAACCTATAGCTGGTACTATCATTATTTATTGACTATATCAAGTTTATAAAGAAGTGCATATCTTTCTGCATCTTATCTCTTATGCTTGT  
ACATTACTGACTCCATAATGTTGAATAGTTGGATATCGACCATGATAGTAATAAATAACTATGATATAGTTCAAATATTTCTTCACGTATAAGAAAGACGTAGAATAGAGAATACGAACA  
T I V S T N C S I L R Y S T S D N N I S V I D L K Y L L A Y E K Q M K D R I S T  
< HIV1 (MN) GP120 GENE

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320  
GGTGATATTGAAAGAGCAGTTTTTCATTCTCTCTCCCTTTATTGTTCCCTCGCTATTACTATTGTTATTAGCAGTACTATTATTGGTATTAGTAGTATTCTCAAATCAGTGCAATTTAA  
CCACTATACTTTCTCGTCAAAAAGTAAAGAGGAGGGGAAATAACAAGGGAGCGGATAATGATAACAATAATCGTCATGATAAACCATAATCATCATAAGGAGTTTAGTCACGTTAAATT  
T I N F S C N K M E G G K I T G E S N S N N H A T S N N T N T T N R L D T C N L  
< HIV1 (MN) GP120 GENE

1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440  
AGTAACACAGAGTGGGGTAAATTTACACATGGCTTTAGGCTTTGATCCCATAACTGATTATATCTCATGCATCTGTCTACCATGTTATTTTCCACATGTTAAAAATTTCTGTAC  
TCATTGTGCTCACCCTCAATTAATAATGTGACCGAAATCCGAAACTAGGGTATTTGACTAATATAGGAGTACGTAGACAAGATGGTACAATAAAAGGTGTACAATTTTAAAGACAGTG  
T V C L P T L K V C P K L S Q D W L S I I D E H M Q E V M N N K W M N F N E T V  
< HIV1 (MN) GP120 GENE

1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560  
ATTTACCAATTTCTACTTCTGTGGGTGGGGTCTGTGGGTACACAGGCATGTGTGGCCCAACATTATGTACCTCTGTATCATATGCTTTAGCATCTGATGCACAAAATAGAGTGGTGGT  
TAAATGGTTAAGATGAAGAACACCAACCCAGACACCCATGTGTCGGTACACACCGGGTTTGTAAATACATGGAGACATAGTATACGAAATCGTAGACTACGTGTTTATCTCACCACCA  
N V L E V E Q P N P D T P V C A H T A W V N H V E T D Y A K A D S A C F L T T T  
< HIV1 (MN) GP120 GENE

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680  
TGCTTCTTTCCACACAGGTACCCCATATAGACTGTGACCCACAATTTTCTGTAGCACTACAGATCATCAACATCCCAAGGAGCATGGTGCCCCATCTCCACCCCATCTCCACAAGTG  
ACGAAGAAAGGTGTGTCCATGGGGTATTATCTGACACTGGGTGTTAAAAAGACATCGTGATGTCTAGTAGTTGTAGGGTTCTCGTACCACGGGGTAGAGGTGGGGGTAGAGGTGTTTAC  
A E K W V P V G Y Y V T V W L K E T A S C I M L M G L L M T G W R W G W R W L H  
< HIV1 (MN) GP120 GENE

1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800  
CTGATATTTCTCCTTCACTCTCATTGCCACTGTCTTCTGCTTTTATATACGATACAACTTAAACGATATCGCGATAATGAAATAATTTATGATTATTTCTCGCTTTCAATTTAACAC  
GACTATAAGAGGAAGTGAGAGTAACGGTGACAGAAGACGAGAAAGTATATGCTATGTTGAATTCGCTATAGCGCTATTACTTTTATAATACTAATAAAGAGCGAAAGTTAAATTTGTTG  
Q Y K E K V R M A V T K Q E K M  
< HIV1 (MN) GP120 GENE

< VACCINIA H6 PROMOTER

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920  
AACCCTCAAGAACCTTTGTATTTATTTTCACTTTTAAAGTATAGAATAAAGAAGCTCTAATTAATTAAGCTACAAATAGTTTCGTTTTCACCTTGCTAATAACTAATTAATTAACCCGG  
TTGGGAGTTCTTGGAAACATAAATAAAGTGAAAAATTCATATCTTATTTCTCGAGATTAATTAATTCGATGTTTATCAAAGCAAAAGTGAACAGATTATTGATTAATTAATTTGGGCC  
< VACCINIA H6 PROMOTER

1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040  
ATCTTGAGATAAAGTAAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAAATCATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAATTAGATCGATGGGAAAAAAT  
TAGAACTCTATTTTCACTTTTATATATAGTAATATAATGTTTCAATGTTAATAAATCCAAATTAGTACCCACGCTCTCGAGTCATAATTCGCCCCCTTAACTAGCTACCCCTTTTTTAA  
> M G A R A S V L S G G E L D R W E K I  
HIV1 (111B) GAG/PRO GENE

2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160  
CGGTTAAGGCCAGGGGGGAAAGAAAAATATAAATTAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCCGAGTTAATCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA  
GCCAATTCGGTCCCCCTTTCTTTTATATTTAATTTTGTATATCATACCGGTCGTCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTCCGACATCTGTT  
R L R P G G K K K Y K L K H I V W A S R E L E R F A V N P G L L E T S E G C R Q  
HIV1 (111B) GAG/PRO GENE

2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280

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## Fig. 7 (cont')

ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAA  
TATGACCCGTGTCGATGTTGGTAGGGAGTCTGTCTAGTCTTCTTGAATCTAGTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTCGTGGTTCCTT  
I L G Q L Q P S L Q T G S E E L R S L Y N T V A T L Y C V H Q R I E I K D T K E  
HIV1 (IIIB) GAG/PRO GENE

2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400  
GCTTTAGACAAGATAGAGGAAGAGCAAAACAAAGTAAGAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTGAGCCAAAATACCTATAGTCAGAACATCCAG  
CGAAATCTGTTCTATCTCTCTCTGTTTTGTTTCATTCTTTTTCTGTGTCGTTCTGTCGTCGACTGTGTCGTTGTCGTTAGTCCAGTCGGTTTTAATGGGATATCAGCTCTTGTAGGTC  
A L D K I E E E Q N K S K K K A Q Q A A A D T G H S N Q V S Q N Y P I V Q N I Q  
HIV1 (IIIB) GAG/PRO GENE

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520  
GGGCAATGGTACATCAGGCCATATCACCTAGAAGTTAAATGCATGGGTAAAGTAGTAGAAGAGAAGGCTTTTCCAGCCAGAGTGATACCCATGTTTTAGCATATCAGAAGGAGCC  
CCCGTTTACCATGTAGTCCGTATAGTGGATCTTGAAATTTACGTACCCATTTTCATCATCTTCTCTTCCGAAAGTCGGGTCTTCACTATGGGTACAAAAGTCGAATAGTCTTCCGCGG  
G Q M V H Q A I S P R T L N A W V K V V E E K A F S P E V I P M F S A L S E G A  
HIV1 (IIIB) GAG/PRO GENE

2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640  
ACCCACAAAGATTTAAACACCATGCTAAACACAGTGGGGGACATCAAGCAGCCATGCAAAATGTTAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCAT  
TGGGGTGTCTAAATTTGTGGTACGATTTGTGTACCCCTCTGAGTTTCGTCGGTACGTTTCAATTTCTCTGGTAGTTACTCTTCGACGCTTACCCATCTCAGCTAGGTACGCTA  
T P Q D L N T M L N T V G H Q A A M Q L K E T I N E E A A E W D R V H P V H  
HIV1 (IIIB) GAG/PRO GENE

2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760  
GCAGGGCTATTGCACAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAAGTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATATCCACCTATCCAGTAGGA  
CGTCCCGGATAACGTGGTCCGGTCTACTCTCTGGTTCCTTCACTGTATCGTCTCTTGGTGAATGATGGGAAGTCTTGTATTCTTACCTACTGTTTATAGGTGGATAGGGTCACTCT  
A G P I A P G Q M R E P R G S D I A G T T S T L Q E Q I G W M T N N P P I P V G  
HIV1 (IIIB) GAG/PRO GENE

2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880  
GAAATTTATAAAGATGGATAATCCTGGGATTAATAAATAGTAAGAAATGTATAGCCCTACCAGCATTCTGGACATAAGACAAGGACCAAAAGAACCCCTTTAGAGACTATGTAGACCGG  
CTTTAAATATTTTCTACCTATTAGGACCCCTAATTTATTTTATCATTTTACATATCGGGATGGTCTGTAAGACCTGTATTCTGTTCTGGTCTTCTGGGAAATCTCTGATACATCTGGCC  
E I Y K R W I I L G L N K I V R M Y S P T S I L D I R Q G P K E P F R D Y V D R  
HIV1 (IIIB) GAG/PRO GENE

2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000  
TTCATAAACTCTAAGAGCCGAGCAAGCTTCACAGGAGGTAAGAAATGGATGACAGAACTTGTGGTCCAAATGCGAAGCCAGATTTGTAAGACTATTTTAAAGCATTGGGACCA  
AAGATATTTGAGATTCTCGGCTCGTTCGAAGTGTCTCCATTTTAACTACTGTCTTTGGAAACCAAGGTTTACGCTTGGGTCTAACATTCTGATAAAATTTTCGTAACCCCTGGT  
F Y K T L R A E Q A S Q E V K N W M T E T L L V Q N A N P D C K T I L K A L G P  
HIV1 (IIIB) GAG/PRO GENE

3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120  
GCGGTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGAGGACCCGCCATAAGGCAAGAGTTTGGTGAAGCAATGAGCCAAGTAACAAATTCAGCTACCATAATGATGCAG  
CGCCGATGTGATCTTCTTACTACTGTCGACAGTCCCTCATCTCTGGGCGGTATTCGGTCTCAAAACCGACTTCGTTACTCGGTTCTTGTAAAGTCGATGGTATTACTACGTC  
A A T L E E M M T A C Q G V G G P G H K A R V L A E A M S Q V T N S A T I M M Q  
HIV1 (IIIB) GAG/PRO GENE

3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240  
AGAGGCAATTTTAGGAACCAAGAAAGATTGTTAAGTGTTCATTTGTGGCAAGAGGACACAGCCAGAAATTCAGGGCCCTAGGAAAAGGGCTGTTGGAATGTGGAAAGGAA  
TCTCCGTTAAATCCTTGGTCTTCTTCAACAATTCACAAAGTTAACACCGTTCTTCCGTTGTGCGGCTTTAACGTCGCGGGATCCTTTTCCGCAACCTTTACACCTTTCCCTT  
R G N F R N Q R K I V K C F N C G K E G H T A R N C R A P R K K G C W K C G K E  
HIV1 (IIIB) GAG/PRO GENE

3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360  
GGACACCAATGAAAGATTGACTGAGAGACAGGCTAATTTTTAGGGAAGATCTGGCTTCTACAAGGGAAGGCCAGGGAATTTCTTCAAGCAGACAGACCAACAGCCCA  
CCTGTGGTTTACTTTCAACATGACTCTGTCCGATTAATAATCCCTTCTAGACCGGAAGGATGTTCCCTTCCGTTCCCTTAAAGAAAGTCTCGTCTGGTCTGGTGTGGGTTGGT  
G H Q M K D C T E R Q A N F L G K I W P S Y K R P G N F L Q S R P E P T A P P  
HIV1 (IIIB) GAG/PRO GENE



## Fig. 7 (cont'd)

3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480  
AAGAGAGCTTCAGGTCTGGGGTAGAGACAACAACCTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTCA  
TTCTCTCGAAGTCCAGACCCCATCTCTGTGTGGAGGGGAGTCTTCCTCCTCGGCTATCTGTTCTTGACATAGGAAATTGAAGGGAGTCTAGTGAGAAACCGTTGCTGGGGAGCAGT  
E E S F R S G V E T T T P P Q K Q E P I D K E L Y P L T S L R S L F G N D P S S  
P Q I T L W Q R P L V  
HIV1 (III B) GAG/PRO GENE

3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600  
AATAAGATAGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCAGGAAGATGGAAACCAAAATGATAGGGGGAATTGGAG  
TTATTTCTATCCCCCGTTGATTTCTTCGAGATAATCTATGTCCTCGTCTACTATGTCATAATCTTCTTACTCAAACGGTCTTCTACCTTTGGTTTTTACTATCCCCCTAACCTC  
Q  
I K I G G Q L K E A L L D T G A D D T V L E E M S L P G R W K P K M I G G I G  
HIV1 (III B) GAG/PRO GENE

3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720  
TTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTC  
AAAATAGTTTCATTCTGTACATAGTCTATGAGTATCTTTAGACACCTGTATTTCGATATCCATGTCATAATCATCTGGATGTGGACAGTTGTATTAACTTCTTTAGACAACCTGAG  
F I K V R Q Y D Q I L I E I C G H K A I G T V L V G P T P V N I I G R N L L T  
HIV1 (III B) GAG/PRO GENE

3730 3740 3750 3760 3770 3780 3790 3800  
GATTGGTTGCACCTTTAAATTTTTAACCCGGGGGATCCCGATTTTATGACTAGTTAATCAAATAAAAGCATACAAGCTATTGCTTC  
CTAACCAACGTGAAATTTAAAAATGGGCCCCCTAGGGCTAAAAATACTGATCAATTAGTTTATTTTTCGTATGTTTCGATAACGAAG  
I G C T L N F < C3 FLANKING ARM  
HIV1 (III B) GAG/PRO

Fig. 8

vcp1452

K3L E3L in C6

10 20 30 40 50 60 70 80 90 100 110  
GAGCTCGCGG CCGCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC TACAAAGGTA TTCATATTTTCT CTATCAATTC TAAAGTAGAT GATATTAATA  
CTCGAGCGCC GCGGATAGT TTTCAGAATT ACTCAATCCA CATCTATCAT ATCTATAATG ATGTTTCCAT AAGTATAAAG GATAGTTAAG ATTTCATCTA CTATAATTAT

120 130 140 150 160 170 180 190 200 210 220  
ACTCAAAGAT GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTGGGACG GTTCACATTT TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA  
TGAGTTTCTA CTACTATCAT CTATTATCTA TCGGAGTATA TTACTGACGT TTAACCTGCG CAAGTGTAAG ATTAGTAGTG CGCAAGTATT CAAAGTTGAC GTATCTAGTT

230 240 250 260 270 280 290 300 310 320 330  
AATCTCACTA AAAAGATAGC CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA TACATAATGG ATTTTGTAT CATCAGTTAT  
TTAGAGTGAT TTTTCTATCG GCTACATAAA CTCTCTCTAA CCTGTAGATT GATGCGATT CTTTAATGTC AATATTTATT ATGTATTACC TAAACAATA GTAGTCAATA

340 350 360 370 380 390 400 410 420 430 440  
ATTTAACATA AGTACAATAA AAAGTATTAA ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCAG ATCTCTCGAG GTCGACGGTA TCGATAAGCT  
TAAATTGTAT TCATGTTATT TTTTATAATT TATTTTATG AATGAATGCT TTTTACTGA TTAATCGATA TTTTGGGTC TAGAGAGCTC CAGCTGCCAT AGCTATTCTGA

450 460 470 480 490 500 510 520 530  
TGATATCGAA TTCATAAAAA TT A TTG ATG TCT ACA CAT CCT TTT GTA ATT GAC ATC TAT ATA TCC TTT TGT ATA ATC AAC TCT AAT CAC TTT  
ACTATAGCTT AAGTATTTTT AA T AAC TAC AGA TGT GTA GGA AAA CAT TAA CTG TAG ATA TAT AGG AAA ACA TAT TAG TTG AGA TTA GTG AAA  
<Q H R C M R K Y N V D I Y G K T Y D V R I V -K  
-----K3L-----

540 550 560 570 580 590 600 610 620  
AAC TTT TAC AGT TTT CCC TAC CAG TTT ATC CCT ATA TTC AAC ATA TCT ATC CAT ATG CAT CTT AAC ACT CTC TGC CAA GAT AGC TTC AGA  
TTG AAA ATG TCA AAA GGG ATG GTC AAA TAG GGA TAT AAG TTG TAT AGA TAG GTA TAC GTA GAA TTG TGA GAG ACG GTT CTA TCG AAG TCT  
<V K V T K G V L K D R Y E V Y R D M H M K V S E A L I A E S  
-----K3L-----

630 640 650 660 670 680 690 700 710  
GTG AGG ATA GTC AAA AAG ATA AAT GTA TAG AGC ATA ATC CTT CTC GTA TAC TCT GCC CTT TAT TAC ATC GCC CGC ATT GGG CAA CGA ATA  
CAC TCC TAT CAG TTT TTC TAT TTA CAT ATC TCG TAT TAG GAA GAG CAT ATG AGA CCG GAA ATA ATG TAG CGG GCG TAA CCC GTT GCT TAT  
<H P Y D F L Y I Y L A Y D K E Y V R G K I V D G A N P L S Y  
-----K3L-----

720 730 740 750 760 770 780 790 800 810  
ACA AAA TGC AAG CAT ACG ATACAACTT AACGGATATC GCGATAATGA AATAATTTAT GATTATTTCT CGCTTTCAAT TTAACACAAC CCTCAAGAAC  
TGT TTT ACG TTC GTA TGC TATGTTTGAA TTGCCTATAG CGCTATTACT TTATTAAATA CTAATAAAGA GCGAAAGTTA AATTGTGTG GGAGTCTGT  
<C F A L M  
-----K3L-----

820 830 840 850 860 870 880 890 900 910 920  
CTTTGTATTT ATTTTCACTT TTTAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGTTT CGTTTTCCCC TTGGCGTATC ACTAATTAAT TAACCCGGGC  
GAAACATAAA TAAAGTGAA AATTTCATAT CTTATTTCTT TCGAGATTAA TTAATTACTT GTCTAACAAA GCAAAAGGGG AACCGCATAG TGATTAATTA ATTGGGCCCG

930 940 950 960 970 980 990 1000 1010 1020 1030  
TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTTCTTT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTAACG GGAACAGGGT TTGTTGATTC  
ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGTGTA TTGGTAATGA TTGCATCTTA CATATCCTTC TCTACATTGC CCTTGTCCCA AACCACTAAG

1040 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140  
GCAAACTATT CTAATACATA ATCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATATAAT TATTTTGTA GATGATGTTA ACTATGTGAT  
CGTTTGATA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATATCT ACGGTTCTAT AGATATATTA ATAAACATT CTACTACAAT TGATACCTA

Fig. 8(cont'd)

1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250  
CTATATAAGT AGTGTAATAA TTCATGTATT TCGATATATG TTCCAACCTCT GTCTTTGTGA TGTCTAGTTT CGTAATATCT ATAGCATCCT CAAAAAATAT ATTGCGATAT  
GATATATTCA TCACATTATT AAGTACATAA AGCTATATAC AAGTTTGAGA CAGAAACACT ACAGATCAAA GCATTATAGA TATCGTAGGA GTTTTTTATA TAAGCGTATA

1260 1270 1280 1290 1300 1310 1320 1330 1340 1350 1360  
ATTCCCAAGT CTTCACTTCT ATCTTCTAAA AAATCTTCAA CGTATGGAAT ATAATAATCT ATTTTACCTC TTCTGATATC ATTAATGATA TAGTTTTTGA CACTATCTTC  
TAAGGGTTCA GAAGTCAAGA TAGAAGATT TTTAGAAGTT GCATACCTTA TATTATTAGA TAAAAATGGAG AAGACTATAG TAATTACTAT ATCAAAAACCT GTGATAGAAG

1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470  
TGTCAAATGA TTCATTATCA CTATATCTAA GAAACGGATA GCGTCCCTAG GACGAACACT TGCCATTAAAT ATCTCTATTA TAGCTTCTGG ACATAATTCA TCTATTATAC  
ACAGTTAACT AAGAATAAGT GATATAGATT CTTTGCCTAT CGCAGGGATC CTGCTTGATG ACGGTAATTA TAGAGATAAT ATCGAAGACC TGTATTAGT AGATAATATG

1480 1490 1500 1510 1520 1530 1540 1550 1560 1570 1580  
CAGAATTAAT GGGAACTATT CCGTATCTAT CTAACATAGT TTTAAGAAAG TCAGAACTCTA AGACCTGATG TTCATATATT GGTTCATACA TGAAATGATC TCTATTGATG  
GTCTTAATTA CCCTTGATAA GGCATAGATA GATTGTATCA AAATCTTCTC AGTCTTAGAT TCTGGACTAC AAGTATATAA CCAAGTATGT ACTTTACTAG AGATAACTAC

1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690  
ATAGTGACTA TTTCATTCTC TGAAAATTGG TAACATCTTC TATATATGCT TTCTTTGTGG ATGAAGGATA GAATATACTC AATAGAATTT GTACCAACAA ACTGTTCTCT  
TATCACTGAT AAAGTAAGAG ACTTTTAACC ATTGAGTAAG ATATATACGA AAGGAACAAC TACTTCTCTAT CTTATATGAG TTATCTTAAA CATGGTTGTT TGACAAGAGA

1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800  
TATGAATCGT ATATCATCAT CTGAAATAAT CATGTAAGGC ATACATTTAA CAATTAGAGA CTTGTCTCCT GTTATCAATA TACTATTCTT GTGATAATTT ATGTGTGAGG  
ATACTTAGCA TATAGTAGTA GACTTTATTA GTACATTCCG TATGTAATTT GTTAATCTCT GAACAGAGGA CAATAGTTAT ATGATAAGAA CACTATTAAA TACACACTCC

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910  
CAAAATTTGTC CACGTTCTTT AATTTTGTTA TAGTAGATAT CAAATCCAAT GGAGCTACAG TTCTTGGCTT AAACAGATAT AGTTTTTCTG GAACAAATTC TACAACATTA  
GTTTAAACAG GTGCAAGAAA TTAAACAAT ATCATCTATA GTTTAGGTGA CCTCGATGTC AAGAACCAGAA TTTGTCTATA TCAAAAAGAC CTTGTTTAAAG ATGTTGTAAT

1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020  
TTATAAAGGA CTTTGGGTAG ATAAGTGGGA TGAATCCTA TTTTAATTA TGTATCGCA TTGCTCTCGT GCAAATATCC AAACGCTTTT GTGATAGTAT GGCATTCAAT  
AATATTTCTT GAAACCCATC TATTCACCCT ACTTTAGGAT AAAATTAATT ACGATAGCGT AACAGGAGCA CGTTTATAGG TTTGCGAAAA CACTATCATA CCGTAAGTAA

2030 2040 2050 2060 2070 2080 2090 2100 2110 2120 2130  
GTCTAGAAAC GCTCTACGAA TATCTGTGAC AGATATCATC TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATTT GTATTTTTTA ATCTATCTCA ATAAAAAAT  
CAGATCTTTG CGAGATGCTT ATAGACACTG TCTATAGTAG AAATCTCTTA TATGATCAGC GCAATTATCA TGATGTAAA CATAAAAAAT TAGATAGAGT TATTTTTTTA

2140 2150 2160 2170 2180 2190 2200 2210 2220 2230  
TAATATGTAT GATTCAATGT ATAACATAAC TACTAACTGT TATTGATAAC TAGAATCA GAA TCT AAT GAT GAC GTA ACC AAG AAG TTT ATC TAC TGC CAA  
ATTATACATA CTAAGTTACA TATTGATTG ATGATTGACA ATAACATTG ATCTTAGT CTT AGA TTA CTA CTG CAT TGG TTC TTC AAA TAG ATG ACG GTT  
<F R I I V Y G L L K D V A L

-----E3L-----

2240 2250 2260 2270 2280 2290 2300 2310 2320  
TTT AGC TGC ATT ATT TTT AGC ATC TCG TTT AGA TTT TCC ATC TGC CTT ATC GAA TAC TCT TCC GTC GAT GTC TAC ACA GGC ATA AAA TGT  
AAA TCG ACG TAA TAA AAA TCG TAG AGC AAA TCT AAA AGG TAG ACG GAA TAG CTT ATG AGA AGG CAG CTA CAG ATG TGT CCG TAT TTT ACA  
<K A A N N K A D R K S K G D A K D F V R G D I D V C A Y F T

-----E3L-----

## Fig. 8 (cont'd)

2330      2340      2350      2360      2370      2380      2390      2400      2410  
AGG AGA GTT ACT AGG CCC AAC TGA TTC AAT ACG AAA AGA CCA ATC TCT CTT AGT TAT TTG GCA GTA CTC ATT AAT AAT GGT GAC AGG GTT  
TCC TCT CAA TGA TCC GGG TTG ACT AAG TTA TGC TTT TCT GGT TAG AGA GAA TCA ATA AAC CGT CAT GAG TAA TTA TTA CCA CTG TCC CAA  
<P S N S P G V S E I R F S W D R K T I Q C Y E N I I T V P N  
-----E3L-----  
2420      2430      2440      2450      2460      2470      2480      2490      2500  
AGC ATC TTT CCA ATC AAT AAT TTT TTT AGC CGG AAT AAC ATC ATC AAA AGA CTT ATG ATC CTC TCT CAT TGA TTT TTC GCG GGA TAC ATC  
TCG TAG AAA GGT TAG TTA TTA AAA AAA TCG GCC TTA TTG TAG TAG TTT TCT GAA TAC TAG GAG AGA GTA ACT AAA AAG CGC CCT ATG TAG  
<A D K W D I I K K A P I V D D F S K H D E R M S K E R S V D  
-----E3L-----  
2510      2520      2530      2540      2550      2560      2570      2580      2590  
ATC TAT TAT GAC GTC AGC CAT AGC ATC AGC ATC CGG CTT ATC CGC CTC CGT TGT CAT AAA CCA ACG AGG AGG AAT ATC GTC GGA GCT GTA  
TAG ATA ATA CTG CAG TCG GTA TCG TAG TCG TAG GCC GAA TAG GCG GAG GCA ACA GTA TTT GGT TGC TCC TCC TTA TAG CAG CCT CGA CAT  
<D I I V D A M A D A D P K D A E T T M F W R P P I D D S S Y  
-----E3L-----  
2600      2610      2620      2630      2640      2650      2660      2670      2680  
CAC CAT AGC ACT ACG TTG AAG ATC GTA CAG AGC TTT ATT AAC TTC TCG CTT CTC CAT ATT AAG TTG TCT AGT TAG TTG TGC AGC AGT AGC  
GTG GTA TCG TGA TGC AAC TTC TAG CAT GTC TCG AAA TAA TTG AAG AGC GAA GAG GTA TAA TTC AAC AGA TCA ATC AAC ACG TCG TCA TCG  
<V M A S R Q L D Y L A K N V E R K E M N L Q R T L Q A A T A  
-----E3L-----  
2690      2700      2710      2720      2730      2740      2750      2760      2770  
TCC TTC GAT TCC AAT GTT TTT AAT AGC CGC ACA CAC AAT CTC TGC GTC AGA ACG CTC GTC AAT ATA GAT CTT AGA CAT TT TTAGAGAGAA  
AGG AAG CTA AGG TTA CAA AAA TTA TCG GCG TGT GTG TTA GAG ACG CAG TCT TGC GAG CAG TTA TAT CTA GAA TCT GTA AA AATCTCTCT  
<G E I G I N K I A A C V I E A D S R E D I Y I K S H  
-----E3L-----  
2780      2790      2800      2810      2820      2830      2840      2850      2860      2870      2880  
CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTATTCA TCATCCTCTG GTGGTTCGTC GTTTCATCG AATGTAGCTC TGATTAACCC GTCATCTATA  
GATTGTGTG GTCGTTATTT TGACTTGGAT GAAATAGTAA AAAAATAAGT AGTAGGAGAC CACCAACCAG CAAAGATAGC TTACATCGAG ACTAATTGGG CAGTAGATAT  
-----E3L-----  
2890      2900      2910      2920      2930      2940      2950      2960      2970      2980      2990  
GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT ATTTCTTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA  
CCACTACGAC CAAGACCTCT AAGACCTCCT CTACCTAATA ATAGACCTTC TTAGAGACAA TAAAGGAACA AAAGTACATA GCTAACGCAA CATGTGAATT CTAACGCTTT  
-----E3L-----  
3000      3010      3020      3030      3040      3050      3060      3070      3080      3090      3100  
TGCTCTAAAT TTGGGAGGCT TAAAGTTTG TTTGCAATCT CTACACGCGT GTCTAACTAG TGGAGGTTCC TCAGCTGCTC TAGTTTGAAT CATCATCGGC GTAGTATTCC  
ACGAGATTTA AACCTCCGA ATTTACAAC AAACGTTAGA GATGTGCGCA CAGATTGATC ACCTCCAAGC AGTCGACGAG ATCAAACCTA GTAGTAGCCG CATCATAAGG  
-----E3L-----  
3110      3120      3130      3140      3150      3160      3170      3180      3190      3200      3210  
TACTTTTACA GTTAGGACAC GGTGTATTGT ATTTCTCGTC GAGAACGTTA AAATAATCGT TGTAACCTAC ATCCTTTATT TTATCTATAT TGATTCTAC TCCTTTCTTA  
ATGAAAATGT CAATCCTGTG CCACATAACA TAAAGAGCAG CTCTTGCAAT TTTATTAGCA ACATTGAGTG TAGGAAATAA AATAGATATA ACATAAGATG AGGAAAGAAAT  
-----E3L-----  
3220      3230      3240      3250      3260      3270      3280      3290      3300      3310      3320  
ATGCATTTTA TACCGAATAA GAGATAGCGA AGGAATTCTT TTTATTGATT AACTAGTCAA ATGAGTATAT ATAATTGAAA AAGTAAATA TAAATCATAT AATAATGAAA  
TACGTAAAAT ATGGCTTATT CTCTATCGCT TCCTTAAGAA AAATAACTAA TTGATCAGTT TACTCATATA TATTAACITT TTCAITTTAT ATTAGTATA TTATTACTTT  
-----E3L-----

*Fig. 8 (cont'd)*

3330 3340 3350 3360 3370 3380 3390 3400 3410 3420 3430  
CGAAATATCA GTAATAGACA GGAAGCTGGCA GATTCTTCTT CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA AAATGATACA GCAAATACAG CTTCAATTCAA  
GCTTTATAGT CATTATCTGT CCTTGACCGT CTAAGAAGAA GATTACTTCA TTATGACGA TTTAGAGGTT TTAATCTATT TTTACTATGT CGTTTATGTC GAAGTAAGTT

3440 3450 3460 3470 3480 3490 3500 3510 3520 3530 3540  
CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA ACTAACTAAG TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT AAAGATTCAT  
GCTTAATGGA AAATTAAGAA AGTCTGTGTG GAATAATGTT TGATTGATTC AGTCTACTAC TCITTCATT ATATTAAAT TGAATACCCA TATTATATTA TTTCTAAGTA

3550 3560 3570 3580 3590 3600 3610 3620 3630 3640 3650  
GATATTAATA ATTTACTTAA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT TCTGGATATT ATAAATACC AGTTAATGAT ATTAATAATAG ATTGTTTAAAG  
CTATAATTAT TAAATGAATT GCTACAATTA TCTGAATAAG GTAGTTGGGG AAGTTTGGA AGACCTATAA TATTTTATGG TCAATTACTA TAATTTTATC TAACAAATTC

3660 3670 3680 3690 3700 3710 3720 3730 3740 3750 3760  
AGATGTAAT AATTATTTGG AGGTAAAGGA TATAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT AATATTAATA ATTATGATAG GAATTTTTTA GGATTTACAG  
TCTACATTTA TTAATAAACC TCCATTTCTT ATATTTTAAT CAGATAGAAA GTGTACCTTT ACTTAATGGA TTATAATTAT TAATACTATC CTTAAAAAAT CCTAAATGTC

3770 3780 3790 3800 3810 3820 3830 3840 3850 3860 3870  
CTGTTATATG TATCAACAAT ACAGGCAGAT CTATGGTTAT GGTAAACAC TGTAACGGGA AGCAGCATTC TATGGTAACT GGCCTATGTT TAATAGCCAG ATCATTTTAC  
GACAATATAC ATAGTTGTTA TGTCCGCTA GATACCAATA CCATTTTGTG ACATTTGCCCT TCGTCGTAAG ATACCATTGA CCGGATACAA ATTATCGGTC TAGTAAATG

3880 3890 3900 3910 3920 3930 3940 3950 3960 3970 3980  
TCTATAACA TTTTACCACA AATAATAGGA TCCTCTAGAT ATTTAATATT ATATCTAACA ACAACAAAAA AATTTAACGA TGTATGGCCA GAAGTATTTT CTAATAATAA  
AGATATTTGT AAAATGTTGT TTATTATCCT AGGAGATCTA TAAATTATAA TATAGATTGT TGTGTTTTT TTAATTTGCT ACATACCGGT CTTCAATAAA GATGATTATT

3990 4000 4010 4020 4030 4040 4050 4060 4070 4080 4090  
AGATAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA GATAATCAIT TAGTAGTAGC TACTAATATG GAAAGAAATG TATACAAAAA CGTGAAGCT TTTATATTAA  
TCTATTTCTA TCAGATAGAA TAGATGTTCT ATACTTTCTT CTATTAGTAA ATCATCATCG ATGATTATAC CTTTCTTAC ATATGTTTT GCACCTTCGA AAATATAATT

4100 4110 4120 4130 4140 4150 4160 4170 4180 4190 4200  
ATAGCATATT ACTAGAAGAT TTAAATCTA GACTTAGTAT AACAAAACAG TTAATGCCA ATATCGATT C TATATTTTAT CATAACAGTA GTACATTAAT CAGTGATATA  
TATCGTATAA TGATCTTCTA AATTTTAGAT CTGAATCATA TTGTTTGTG AATTACCGT TATAGCTAAG ATATAAAGTA GTATTGTCAT CATGTAATTA GTCATATAT

4210 4220 4230 4240 4250 4260 4270 4280 4290 4300 4310  
CTGAAACGAT CTACAGACTC AACTATGCAA GGAATAAGCA ATATGCCAAT TATGTCTAAT ATTTTAACCT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG  
GACTTTGCTA GATGTCTGAG TTGATACGTT CCTTATTCGT TATACGGTTA ATACAGATTA TAAATTGAA ATCTTGATT TCGAAGATGG TTATGATTTT TATCCTATGC

4320 4330 4340 4350 4360 4370 4380 4390 4400 4410 4420  
TGATAGGCTG TTAAGAGCTG CAATAAATAG TAAGGATGTA GAAGAAATAC TTTGTCTAT ACCTTCGGAG GAAAGAACTT TAGAACAACCT TAAGTTTAAAT CAACTTGTA  
ACTATCCGAC AATTTTCGAC GTTATTTATC ATTCCTACAT CTTCTTTATG AAACAAGATA TGAAGGCTC CTTTCTTGAA ATCTTGTTGA ATTCAAATTA GTTTGAACAT

4430  
TTTATGAAGG TACC  
AAATACTTCC ATGG

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/02669**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12N 15/64, 15/67, 15/86; A61K 48/00

US CL : 424/93.2, 199.1; 435/69.1, 172.3, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 199.1; 435/69.1, 172.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS; DIALOG DATABASES: MEDLINE, BIOSIS, CA SEARCH, WORLD PATENT INDEX**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| A         | US 4,738,922 A (HASELTINE et al.) 19 April 1988, see entire document.   | 1-14, 18-26           |
| A         | PARK, H. et al. TAR RNA-binding protein is an inhibitor of the interferon-induced protein kinase PKR. Proc. Natl. Acad. Sci. USA. May 1994, Vol. 91, pages 4713-4717. See entire article. | 1-14, 18-26           |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

02 APRIL 1998

Date of mailing of the international search report

20 JUL 1998

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